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PLANT PHYSIOLOGY

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PHOTOSYNTHESIS AND ABSORPTION OF MINERAL NUTRIENTS AND WATER BY PLANT ROOTS

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Photosynthesis and root nutrition are the processes furnishing materials and energy for life activity and growth of plants. The size and quality of harvests depend on the course and intensity of the nutritive processes. The photosynthesis and root nutrition in these processes represent an intact system and cannot be evaluated separately for development of measures to increase productivity. Large harvests cannot be obtained without providing proper mineral nutrition and good photosynthetic environments.

Absorption, transmission and assimilation of elements require expenditure of energy. The only energy source in plants is photosynthesis. The determining stage of root nutritive processes is not only the absorption, but the assimilation of mineral nutrients, i.e., in a number of cases their inclusion in the composition of organic compounds and involvement in metabolism of organic substances. However, the primary source of the latter is also photosynthesis. In other words, in the absence of or with weak photosynthesis no intensive assimilation of mineral nutrients is possible.

These general aspects are confirmed by the experimental data of many investigators [1-5], who showed a direct relationship between the assimilation rate of mineral nutrients and the presence or absence of light (and, consequently, also of photosynthesis) either under natural or by artificially substituted conditions.

This was shown especially clearly in relation to nitrate assimilation [6-11]. A number of the cited workers relate the better nitrate assimilation in light to the fact that, apart from their customary reduction in darkness, there exists also a specific photochemical reduction, closely related to the activity of the photosynthetic mechanism.

In a number of studies [12-14] other mechanisms are also revealed of interaction between the two processes of plant nutrition. In particular, a direct relationship was established between respiration and the rate of assimilation by roots of mineral nutrients. Based on such comparisons, a theory is advanced as to assimilation of mineral nutrients on the basis of roots interchanging HCO_3^-, H^+ and organic acid anions for mineral nutrients salt ions [15-19].

In other words, from these studies a theory evolves of a relationship between photosynthesis and mineral nutrition by means of the respiration process.

The studies of A. L. Kursanov and collaborators on transfer of assimilation products and the specific roots activities [20-25] have a special significance from the point of view of understanding the mechanism of interrelation between photosynthesis and mineral nutrition.

In these studies such facts were established as the effective rapid flow of photosynthetic products (sucrose, oligosugars) into roots, incorporation of these products in the roots by Krebs cycle conversions, as a result of which acceptors are formed for assimilation of ammonia nitrogen with formation of amino acids.

Thus, important data have already been obtained characterizing the relation between photosynthesis and root nutrition, as well as the essentials of the mechanisms of these relationships. The course of further development of these studies was also determined.

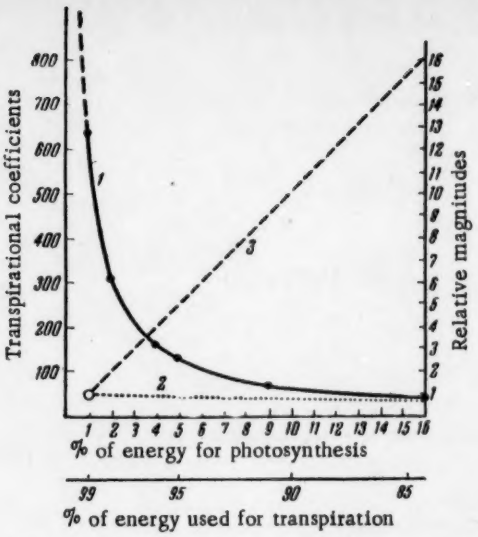

Fig. 1. Curves of possible variations in the size of transpirational coefficients (1) transpirational intensity (2) and accumulation of dry biomass (3) relative to the percentage of the absorbed radiation energy utilized for photosynthesis.

However, there are almost no practical studies which emphasize the quantitative side of these relationships, while from both the theoretical and practical points of view this question appears to be very important for further development of theories and practice for obtaining large harvests.

To obtain large harvests of agricultural plants it is necessary to have biological yields of 12-18 tons of total dry biomass. In such cases the plants must assimilate and tolerate such large quantities of nutrients as 200-300 kg nitrogen and potassium and 100-150 kg phosphorus [26, 27].

At the same time, a large yield is possible only with good structure of plants, at a very good development of a leafy area (not less than 40 thousand m^2 per hectare at the maximum growth period); but with an increase of the leaf area in plantings the degree of their reciprocal shading is increased, and related to this the photosynthetic conditions are worsened, and the intensity and productivity of this process decreases [28-31]. This, in turn, can decrease assimilation of mineral nutrients.

It is true that high fertility backgrounds, in their turn, can activate photosynthetic activity and produce a sufficiently high photosynthesis even when the leaf area is greatly developed.

Based on this reciprocal support of photosynthesis by mineral nutrition and of the latter by photosynthesis, there is evidently a good possibility of increasing plant productivity. This is shown by the yields obtained in the Chinese Peoples' Republic [32] by the combined utilization of large doses of fertilizers and irrigation on plantations of great density. However, this question has been hardly studied and requires urgent attention.

Of no less significance also is the question of the correlation and reciprocal effects of photosynthesis and the water supply of plants.

The driving force of the photosynthetic and transpiration processes is the energy of sun radiation absorbed by leaf chlorophyll, though of absorbed energy of the visible spectrum only 1-2% is used for photosynthesis, the remaining 99-98% being converted into heat and resulting in water evaporation. It is very clear that the more energy absorbed by the leaves is utilized for photosynthesis, the more organic substances will be produced in the harvest; however, less energy will be consumed in water evaporation. Related to this, the transpirational coefficients will decrease and the productivity of moisture will increase.

TABLE 1

Assimilation of Nitrates by Plant Roots in Light and Darkness (3-hour exposure; NO_3^- in the medium 0.383 mgm)

Expt. No.	NO_3^- assimilated in light			NO_3^- assimilated in darkness		
	total mg	per g raw root weight	mean	total mg	per g raw root weight	mean
1	0.123	0.123	0.109	0.058	0.058	0.049
2	0.070	0.070		0.037	0.037	
3	0.116	0.116		0.060	0.060	
4	0.190	0.127		0.052	0.043	

TABLE 2

Assimilation of Ammonia by Plant Roots in Light and Darkness (2-hour exposure; initial NH_4^+ in medium 0.1390 mg)

Expt. No.	NH_4^+ assimilated in light			NH_4^+ assimilated in darkness		
	total mg	per g raw root weight	mean	total mg	per g raw root weight	mean
1	0.0726	0.1016	0.0985	0.0180	0.0252	0.0289
2	0.0674	0.1026		0.0161	0.0225	
3	0.0629	0.1021		0.0405	0.0567	
4	0.0549	0.0878		0.0065	0.0104	

It should be noted that there can be considerable variation in the cited indices. Thus, for instance, the utilization of sun energy radiation absorbed by the leaves on photosynthesis is 1-2%. In relatively good cases it reaches 5-6 or even 8-10% [30-33]. There are indications in the literature that plantings have utilized even 20-25% of sun radiation energy for photosynthesis. In such cases the percentage of energy absorbed by leaves for transpiration must decrease in a range from 99 to 75%.

Taking into consideration that the use for transpiration of each 586 large calories must evaporate 1 l of water, and the use for photosynthesis of each 112 large calories must assimilate 44 g of CO_2 and form 30g of photosynthetic products, the results of such differential distribution of absorbed energy for photosynthesis and transpiration can be depicted graphically. From the plotted data (Fig. 1) it can be seen that by increasing photosynthetic productivity or (the equivalent) the percentage of sun energy radiation absorbed by leaves for photosynthesis and "diverting" it from transpiration, the yield may be greatly increased (by several times) with the same expenditure of water for transpiration as before, or even at some lesser expenditure. As indicated, the question of correlation between photosynthetic mineral nutrition and transpiration is significant not only in theory but in practice and, therefore, requires detailed study. This impelled us to begin a series of experiments for studying the indicated questions. Some results of these studies are given below.

METHODS

Nagrada variety of corn was used. The experimental plants were first grown in sand. After removing the plant endosperm when 16-18 days old they were transferred to 1:4 liquid Hellriegel solution.

TABLE 3

Assimilation of Nitrates by Plant Roots in Light and Darkness without CO_2 (3-hour exposure; initial NO_3^- in the medium 0.383 mg)

Expt. No.	NO_3^- assimilated in darkness without CO_2			NO_3^- assimilated in darkness without CO_2		
	total mg	per g raw root weight	mean	total mg	per g raw root weight	mean
First experimental series						
1	0.090	0.100	0.082	0.097	0.104	0.081
2	0.0630	0.070		0.072	0.080	
3	0.0692	0.078		0.054	0.060	
Second experimental series						
1	0.1170	0.130	0.098	0.0891	0.099	0.078
2	0.0608	0.098		0.0608	0.098	
3	0.0424	0.053		0.0232	0.029	
4	0.1140	0.114		0.0770	0.077	

Note to Tables 3 and 4. In the first experimental series CO_2 free air was constantly blown through, and glasses with 33% KOH were placed in the experimental chambers. In the second series of experiments CO_2 free air was blown through, but no glasses with 33% KOH were placed in the experimental chambers.

TABLE 4

Assimilation of Ammonia by Plant Roots in Light and in Darkness without CO_2 (2 hour exposure; initial NH_4^+ in medium - 0.1390 mg)

Expt. No.	NH_4^+ assimilated in light without CO_2			NH_4^+ assimilated in darkness without CO_2		
	total mg	per g raw root weight	mean	total mg	per g raw root weight	mean
Second series of experiment						
1	0.0336	0.0420	0.0384	0.0324	0.0405	0.0403
2	0.0324	0.0360		0.0259	0.0288	
3	0.0371	0.0371		0.0519	0.0519	
Third series of experiments						
1	0.0270	0.0270	0.0262	0.0138	0.0138	0.0157
2	0.0216	0.0259		0.0096	0.0166	
3	0.0216	0.0259		0.0096	0.0166	

The experiments were designed to study the significance of photosynthesis in assimilation of nitrate and ammonia ions. For this purpose potassium nitrate and ammonium sulfate were added to the nutrient media in approximately 0.0002 N concentration. The pH of nutrient mixtures was adjusted by adding appropriate quantities of dilute acid (HCl) and dilute alkali (NaOH); in experiments with KNO_3, pH = 5.8, while in experiments with $(NH_4)_2SO_4$ = 6.8. Illumination was provided by a reflector lamp. Illumination intensity was 3500 lux.

For experiments in darkness the experimental plants were placed in a dark chamber. In experimental study of effects of light without CO_2 the plants were placed in hermetically sealed plexiglas chambers, in which glasses with 33% KOH were placed, and air, previously freed from CO_2, was continually blown through. In some experiments only air freed from CO_2 was blown through, but without a solution of alkali in the chamber.

The experiments were conducted at room temperature.

Absorption of studied substances was determined photocolorimetrically by variations of NO_3^- and NH_4^+ concentrations in the external solution. Determination of NO_3^- was by the Granval-Liègeois method, NH_4^+ determination with Nessler's reagent. Variations in root respiration were determined by blowing air through the nutrient mixture absorbing CO_2 by alkali and subsequent filtration. Rate of transpiration was determined by a transpirometer. Of many seedlings, plants having similar root dimensions were selected for the experiment.

Assimilation of nutrients during the exposure (usually 2-3 hours) was calculated in percentages of the original quantity of the given ion (NO_3^- and NH_4^+) in the initial solution and per mg of fresh root weight.

RESULTS AND DISCUSSION

One of the questions studied was the effect of light and darkness on nitrogen assimilation by the plant roots. Data obtained in the first experimental series are given in Tables 1-2.

Data in Table 1 and 2 coincide with those of other authors and show that assimilation of nitrogen from nitrates, as well as from ammonia, is considerably more intense in light. However, in our experiments it is shown that the degree of reaction to light of the assimilation process is dissimilar in nitrate and ammonia nitrogen and is considerably greater in the case of ammonia nitrogen.

Before evaluating the results of these experiments, we shall state the data of the second series, when the rate of nitrate and ammonia nitrogen assimilation was recorded in relation to the presence of light in the absence of CO_2. In these cases with "light + CO_2" (the first series of experiments) we dealt with the real photosynthesis, while with "light − CO_2" only with the possibility of proceeding through a photochemical reaction (as, for example, assumed reduction of nitrates), but in the absence of true photosynthesis. Results of these experiments are given in Tables 3 and 4.

TABLE 5

Respiration of Plant Roots in Nutrient Solutions of KNO_3 and $(NH_4)_2SO_4$ in Light and in Darkness (mg CO_2 liberated by roots per g dry weight per hour 3-hour exposure)

Expt. No.	Light + CO_2*	Darkness + CO_2	Light without CO_2	Darkness without CO_2
0.0002 N KNO_3 solution				
1	1.162	0.237	0.356	0.309
2	0.767	0.318	0.523	0.455
3	0.878	0.466	—	—
4	0.103	0.087	—	—
Mean	0.728	0.277	0.419	0.382

Expt. No.	Light + CO_2*	Darkness + CO_2	Light without CO_2	Darkness without CO_2
0.0002 N $(NH_4)_2SO_4$ solution				
1	2.596	0.347	0.891	0.809
2	1.127	0.372	0.508	0.609
3	1.170	0.340	—	—
Mean	1.631	0.353	0.699	0.706

*In the course of the experiment air free of CO_2 was continually blown through and glasses with 33% KOH solution were placed into experimental chambers.

As seen from comparisons of Tables 1-2 and 3-4 (the first and second series of experiments), the stimulatory effect of light on nitrate and ammonia nitrogen assimilation is reflected only with "light + CO_2", i.e., in presence of true photosynthesis. This is confirmed also by the third series of experiments, when only air free of CO_2 was blown through chambers without KOH solution. In these experiments reutilization of respiratory carbonic acid was possible for photosynthesis.

Related to this there was a positive effect of light on nitrogen assimilation, especially in cases of NH_4^+ assimilation, although less than in the first series of experiments.

The following conclusions can be made from the three series of experiments: light has a positive effect on nitrate and ammonia nitrogen assimilation in the presence of true photosynthesis; the strongest positive effect of the photosynthetic presence is manifested in assimilation of ammonia nitrogen.

A lesser positive reaction of photosynthesis in assimilating nitrates is evidently related to the necessity for consuming more energy and materials for their reduction as oxidixing compounds.

The "photochemical reduction of nitrates", stated by a number of authors, should be interpreted not as a result of an independent photochemical reaction, unrelated to photosynthesis, but as a process closely related to photosynthesis. Most probably in the process of photosynthesis early products of CO_2 reduction are formed which are active nitrate reducers. This position is confirmed by results of a study by Andreeva [14], who established that reduction of nitrates in light by leaves rich in or enriched by carbohydrates, but deficient in CO_2, occurs less intensely than in leaves of "light + CO_2".

At the same time such facts as a more intense reduction and assimilation of nitrates in blue, by comparison with red light [9], signify that in reduction and assimilation of nitrates an important role is also played by additional photochemical reactions (possibly activating cytochrome and flavin oxidase systems) which assist energy utilization of active early photosynthetic products mentioned above in reducing nitrates or nitrites [10].

As concerns ammonia nitrogen, its assimilation is accomplished more easily without those additional outlays in material and energy which are needed for nitrate assimilation, and therefore the positive reaction on photosynthesis in its assimilation is especially precise and intense. In this case, the basic positive role, evidently, is played by the intensified formation of the usual photosynthetic products in the photosynthetic process – those of carbohydrates. The positive role of their presence in plants in assimilation of ammonia nitrogen was noted even in Pryanishnikov's studies [34]. In addition, the ammonia nitrogen is probably more active than nitrate nitrogen in the sense of effecting transmission of photosynthetic products to the roots. Such a conclusion can be made from the experimental results presented in Table 5.

TABLE 6

Intensity of Plant Transpiration with Presence or Absence of Photosynthesis (nitrogen in the form of NO_3^-; 2-hour exposure)

Expt. No.	Light			Light without CO_2			Darkness		
	loss of water in the apparatus in ml/2hr	leaf area in cm^2	transpiration in ml/in^2	loss of water in the apparatus in ml/in^2	leaf area in cm^2	transpiration in ml/in^2	loss of water in the apparatus in ml/in^2	leaf area in cm^2	transpiration in ml/in^2
1	0.151	55.2	0.271	0.213	47.0	0.453	0.060	41.8	0.125
2	0.119	79.2	0.150	0.240	79.2	0.303	0.135	79.20	0.170
3	0.317	79 0	0.401	0.412	79.0	0.521	0.210	79.00	0.266
4	0 107	47.8	0.204	0.249	60.4	0.414	0.045	42.8	
Mean	—	—	0.213	—	—	0.423	—	—	0.167

In these experiments the effect of photosynthesis on root respiration was studied. This effect was found to be clearly positive and especially strong in the variant "light + CO_2" and in the presence of $(NH_4)_2SO_4$ in the nutrient medium. In variants without CO_2, light, even in presence of $(NH_4)_2SO_4$ in the nutrient medium, did not have a positive effect on root respiration.

Thus, the effect of photosynthesis on assimilation of mineral nutrients is accomplished, first of all, through the process of transmitting assimilates into roots and their conversion in the respiratory cycle in accordance with facts established by studies of Kursanov et al. [20-25].

However, this is not the only aspect of the mechanism relating photosynthesis and mineral nutrition. In a number of cases, as, for instance, in assimilating nitrates (and probably sulfates) reduction processes are closely and locally related to the photosynthetic process and the photosynthetic mechanism.

Aside from the usual dark path of reduction, it is also accomplished as part of the photosynthetic process. In this case, according to several authors' hypotheses [35], their reduction may even constitute a rivalry to CO_2 photosynthetic reduction. Therefore, the presence of photosynthesis possibly exerts a lesser effect on acceleration of nitrate assimilation by roots than of ammonia nitrogen.

It should be noted that differences in the effect of photosynthesis on assimilation of ammonia and nitrate nitrogen can evidently be especially marked in relatively weak light (in out experiments its intensity reached over 3500 lux), and here also possible competition between CO_2 and NO_3^- for the reducing power of photosynthesis can be especially intense.

In strong light and at a high photosynthetic level there may be less reason for competition: intense light can secure a full measure of one, as well as the other process, and the difference in positive effect of light on assimilation of ammonia and nitrate nitrogen may be small or simple nonexistent. This hypothesis requires experimental verification, but we considered it necessary to mention it here to forewarn against a possible positiveness of the above stated conclusions.

Finally, let us examine data on the effect of photosynthetic conditions on plant transpiration (Table 6), citing only experimental data with nitrates as the nitrogen source. Similar data were obtained with ammonia nitrogen. As seen from data in Table 6, presence of photosynthesis substantially diminishes transpiration intensity. However, it should be remembered that our experiments were conducted under artificial, fairly weak light, with an increased content of CO_2 in the air, where the precentage of light utilization for photosynthesis is usually high. At a high light intensity, where the proportion of light utilization for photosynthesis is ordinarily low, the effects of photosynthetic influence on transpiration intensity should be less. But the effect of photosynthetic intensity on transpiration coefficients and on transpiration productivity can be very strong. Thus, for example, an increase in photosynthetic intensity and the coefficient of light energy utilization for photosynthesis from 1 to 2.4% and more (which is fully attainable in practical work) will diminish transpiration intensity comparatively little. But since a two- or threefold increase in the coefficient of light energy utilization for photosynthesis signifies a corresponding increase in production of organic mass during harvests, in such cases the transpirational coefficients must decrease and indices of transpiration productivity must increase correspondingly (see Fig. 1).

SUMMARY

In the first stage of our investigation of the influence of photosynthesis on root nutrition some experiments were carried out in which the effect of photosynthesis (light + CO_2) or absence of photosynthesis (darkness or light $-CO_2$) on the absorbing power of roots was studied in comparatively short exposures. The experiments indicated that such processes as nitrate uptake, ammonium ion uptake, respiration and transpiration rapidly respond (during 2-3 hours) to the presence or absence of photosynthesis.

The rate of this response is comparable with the rate of movement of assimilates from leaves to the roots as found by A. L. Kursanov and co-workers.

Uptake of NO_3^- and NH_4+ sharply differ from each other in respect to their dependence on photosynthesis and this signifies that the mechanism of uptake of various nutrients is different.

Photosynthesis significantly lowers the transpiration rate probably as a result of use of part of the energy absorbed by the leaves in photosynthesis and hence decrease of it in transpiration.

The results of these preliminary experiments show that the problem of the interrelationship between photosynthesis and root nutrition of plants is of great importance and deserves further study.

In further work on raising crop productivity special attention should be paid to the problem of raising the photosynthetic activity of plants as it is this property which determines to a great extent the efficiency of plants in respect to use of soil fertility. In this case breeding of plants with enhanced photosynthetic activity should be used parallel with application of agrotechnical and agrophysiological methods.

LITERATURE CITED

[1] N. G. Potapov and N. Z. Stankov, Doklady Akad. Nauk SSSR 2, 1, 40 (1934).

[2] N. G. Potapov, Vestnik Agrotekh. VASKhNIL 2, 71 (1940).

[3] T. C. Brover, Plant Physiol. 25, 367 (1950).

[4] A. H. Burg, Influence of light on the absorption of potassium by maize incarbon dioxide–free air, Proc. Koninkl. Acad. Wet ser. C, 55 (1952).

[5] R. J. Helder, Acta Bot. neerl. 1, 3, 362 (1952).

[6] H. Burstrom, Ann. Agr. Coll. Sweden 11, 1 (1943).

[7] H. Burstrom, Arkhiv Bot. A 30, 8, 1 (1943).

[8] H. Burstrom, Nitrate reduction," Radiation Biology, Ed. by Hollaender. (Mc Graw Hill Book 1957).

[9] V. Stoy, Physiol. Plantarum 8, 963 (1955).

[10] E. Kessler,"Contributions to the problem of photochemical nitrate reduction," Research in Photosynthesis, Ed. by H. Gaffron. (Intersc. Publ. Inc., N. Y., 1957).

[11] T. F. Andreeva, Doklady Akad. Nauk SSSR 78 (5), 1033 (1951).

[12] H. Jenny and E. Cowan, Science 77, 394 (1933).

[13] D. Hooland and T. Broyer, Plant Physiol. 11, 471 (1936).

[14] E. Steward, R. Berry and T. B oyer, Ann. Bot. 50, 1 (1936).

[15] F. Steward, P. R. Stout and C. Preston, Plant Physiol. 15, 409 (1940).

[16 H. Lundegardh and H. Burstrom, Biochem. Z. 261, 235 (1943).

[17 H. Lundegardh, Biochem. Z. 290, 104 (1937).

[18] D. A. Sabinin, Mineral Plant Nutrition [In Russian]. Izd. AN SSSR (1940).

[19] D. A. Sabinin, Physiological Basis of Plant Nutrition [In Russian]. Izd. AN SSSR (1955).

[20] A. L. Kursanov, Bot. Zhur. 39, 482 (1954).

[21] A. L. Kursanov, Voprosy Bot. 1 (1954); Izv. Akad. Nauk SSSR, ser. biol. 6, 698 (1957).

[22] A. L. Kursanov, M. Kh. Chailakhyan, O. A. Pavlinova, M. V. Turkina and M. I. Brovchenko, Fiziol. Rastenii 5, 3 (1958).*

[23] O. N. Kulaeva, E. I. Silina and A. L. Kursanov, Fiziol. Rastenii 4, 6 (1957).*

[24] N. A. Pristupa and A. L. Kursanov, Fiziol. Rastenii 4, 417 (1957).*

[25] N. A. Pristupa, Fiziol. Rastenii 6, 30 (1959).*

[26] A. A. Nichiporovich, "Photosynthesis and the theory of obtaining large harvests" [in Russian] Timiryazev Lecture XV. Izd. AN SSSR (1956).

[27] A. A. Nichiporovich and L. E. Stroganova, Agrochimica (Italia) 2, 1, 27 (1957).

[28] D. J. Watson, "Leaf growth in relation to crop yield" Growth of Leaves, Ed. by F. L. Milthrope (London, 1956).

[29] D. J. Watson, Ann. Bot. n. s. 22, 37 (1958).

[30] G. E. Blackman and J. N. Black, Ann. Bot. 23, 131 (1959).

[31] L. E. Stroganova, Studies of Conference II on Photosynthesis [in Russian] (Izd. AN SSSR, 1959).

[32] In'Hung-chang et al. Scientific News, Academy of Sciences of Chinese Peoples Republic 18, 566 (1958).

[33] A. A. Nichiporovich and S. N. Chmora, Fiziol. Rastenii 5, 320 (1958).*

[34] D. N. Pryanishnikov, Ammonia, Nitrates, and Nitrites as Nitrogen Sources for Higher Plants. Selected Studies, Vol. I (Izd. AN SSSR, 1951).

[35] A. Moiz, Fiziol. Rastenii 6, 274 (1959).*

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EFFECT OF AERIAL PLANT ORGANS ON P^{32} UPTAKE BY THE ROOT SYSTEM

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In relation to the established intense synthetic activity of the root system [1-3], there has of late been an increased interest in the interdependence of activity of individual plant organs [3-5], and primarily in the interdependence between roots and aerial organs, which should bring a better understanding of the processes of the plant organism's life activity.

Our investigations were set up to study the simplest phenomena, namely: the absorption by the root system of nutrient elements from surrounding solutions for the intact plant, and for the root system alone.

With the isotope method the relative assimilation intensity of any element by the root system can be accurately established from its presence in the plant's aerial organs.

Prior to development of the isotope method, the activity of the root system after cutting of aerial organs was considered within known limits as a reflection of the normal root activity of the total plant, and the data of sap analysis, obtained after cutting the stem, served as an evaluation of the plant's quantitative supply of nutrient elements [4,6].

In the experiments corn plants were used, cultivated for a month in aqueous cultures at two levels of phosphorous nutrition—0.03 and 0.015 g P_2O_5 per liter of nutrient solution, in order to clarify the effect of the plants' phosphorus supply on its subsequent assimilation. The other elements were introduced on the basis fo the following quantities per 1 of nutrient mixture: N 0.100 g, K_2O 0.077 g, MgO 0.032 g, CaO 0.100 g, iron citrate 6 mg, H_3BO_3 1 mg, and $MnCl_2 \cdot H_2O$ 3 mg.

The difference between plants supplied with full and half-strength phosphorus doses were comparatively small, as shown by subsequent calculations of their dry mass (Table 1).

The experiment was conducted as follows: three corn plants were used in each of 4 parallel vessels. After cutting aerial organs of the first plant in each vessel, a collecting vessel was attached to each stump to collect sap and immediately 32.2 μC of P^{32} was added to the 2.6 l of solution in each vessel. After 24 hours the collecting vessel was removed for measurement and analysis of the sap and another collecting vessel was attached to the stump. At the same time the aerial portion of the second plant was cut to compare the P^{32} uptake during the first 24 hours by the aerial portion of this plant and that excreted by the sap of the first plant.

Subsequently the sap from the first plant was collected for another 48 hours and at the end of this period the aerial portion of the third plant was cut so as to establish any differences in P^{32} absorption for 72 hours by the root system of the whole plant and the plant with cut aerial portion. Finally from the third plant the sap was collected for another 24 hours.

The P^{32} uptake was recorded separately for each plant, and the data given in the Tables are the mean for all four vessels. The activity of all determinations was calculated to the day when P^{32} was added to the nutrient solutions.

TABLE 1

Influence of Intensity of Plants' Phosphorus Nutrition on Dry Mass Formation and Phosphorus Content in Corn (mean of 12 plants)

Phosphorus dosage	Dry mass of single plant, in g		P_2O_5 content, mg/g*	
	aerial organs	roots	aerial organs	roots
1.0	5.03	1.50	16.0	18.0
0.5	4.64	1.54	13.2	14.8

*Differences in phosphorus content were greater.

The degree to which plants were supplied with phosphorus preceding their inclusion in the experiment had a substantial effect on P^{32} assimilation by the whole plant (Table 2).

Plants which received a half dose of phosphorus before the experimental period assimilated twice as much P^{32} during the experiment as plants which received a full dose of phosphorus before the experiment.

The sap was collected from the first plant from the moment P^{32} was added, and from the third plant after a lapse of 72 hours. In the variant with half doses of phosphorus the intensity of sap excretion by two plants increased greatly, and therefore the mean values were calculated separately for each pair of plants (Table 3).

TABLE 2

P^{32} Uptake into Aerial Corn Organs (pulses/minute plant)

Phosphorus dosage	Plant No.	Uptake for	
		24 hours	72 hours
1.0	2	342,400	-
	3	-	888,410
0.5	2	768,400	-
	3	-	1,720,000

Moreover, it was observed that plants better supplied with phosphorus excreted less sap.

Plants grown before the experiment on a lower phosphorus dosage excreted much more radioactive phosphorus in the sap than plants which earlier received a full dosage of phosphorus (Table 4).

Thus, conclusions arrived at by data of sap analysis coincide with the conclusions based on analysis of aerial plant mass (Table 2).

However, in comparison of absolute amounts of P^{32}, it can be seen that the root system deprived of aerial organs is much weaker. For the first 24 hours plants No. 1 excreted almost 1/40 the P^{32} in the sap of the uptake for the same period into aerial organs of plants No. 2, and the difference increased further over the 72-hour period.

Thus, absolute amounts of P^{32} excreted by the sap of roots with aerial organs cut and amounts assimilated by the whole plant are not comparable. In the given case a relatively long period was taken for sap measurements 24 hours. It is necessary to verify the differences observed in shorter periods. However, even the results so far

TABLE 3

Sap Excretion by Corn Plants (ml per plant)

Phosphorus dosage	Plant No.	First 24 hours	Next 48 hours	Total for 72 hours
1.0	1	2.36	0.59	2.05
	3	6.67	-	-
0.5	1	2.69	1.19	3.88
	1	8.86	6.43	15.29
	3	8.89	-	-

obtained indicate that a careful approach in treating data obtained by the sap method is required. Evidently, these data can serve for relative evaluation of root system activity in different environments, but not for obtaining absolute figures in supplying plants with nutrients.

As can be seen from data in Table 4, the total P^{32} excreted by sap is diminished in time, but the P^{32} concentration in the collected sap is increased.

Attention is called to the great variation of P^{32} excreted in the sap for the first 24 hours by plants No. 1 and No. 3. Evidently, leveling of the isotope phosphorus composition in the sap requires some time, and the intensity of P^{32} excretion in the sap gradually decreases in the process of plant nutrition by this isotope until it reaches a definite level, related to the plant requirements, the concentration and the isotope composition in the medium. Therefore, plants No. 3, receiving P^{32} for 72 hours before cutting aerial organs and used for obtaining sap only after this period, already had a more fixed P^{32} content in the sap furnished by the root system.

That a definite time and a constant supply of radioisotopes are required for supporting normal P^{32} content in sap moving from the root system into the aerial organs is also indicated by the experiments in which in one of the variants P^{32} temporarily was eliminated from the nutrient solution [7], which markedly diminished P^{32} uptake into aerial organs.

Simultaneously with measuring amounts of P^{32}, analyses were conducted for the content of total phosphorus in the sap. In contrast with P^{32} measurement, these analysis did not show any greater phosphorus assimilation for the first 24 hours by plants cultivated on a poor phosphorus background (Table 5).

For the next 48 hours an uptake of even greater intensity is observed of phosphorus into plants which received less phosphorus before the beginning of the experiment, but this occurs chiefly at the expense of a marked decrease of phosphorus assimilation by plants from a richer background.

TABLE 4

P^{32} Content in Corn Sap

Phosphorus dosage	Plant No.	P^{32} excreted with sap, pulses/minute · plant			P^{32} concentration in sap, pulses/min · g	
		first 24 hr	next 48 hr	total for 72 hr	first 24 hr	next 48 hr
1.0	1	9329	4 087	13 416	3 810	9 243
	3	129 558	—	—	19 314	—
0.5	1	23 737	21 000	44 737	11 022	14 764
	1	293 554	454 160	748 214	32 974	77 382
	3	280 125	—	—	31 528	—

TABLE 5

P_2O_5 Content in Corn Sap

Phosphorus dosage	Plant No.	Excreted with sap, mg per plant			P^{31} concentration in sap, mg/g	
		first 24 hr	next 48 hr	total 72 hr	for 24 hr	for 48 hr
1	1	0.269	0.056	0.325	0.133	0.046
	3	2.251	—	—	0.337	—
0.5	1	0.217	0.168	0.385	0.107	0.135
	1	1.405	3.118	4.423	0.161	0.518
	3	2.421	—	—	0.272	—

Variations in radiophosphorus concentration with time and in experimental variants were much more intense than variations in content of ordinary phosphorus (Tables 4 and 5).

Data shown in Table 5 usually were found when the sap method was used in earlier investigations, before the discovery of the isotope method of analysis.

Variations in data on P^{32} and P^{31} assimilation given in Tables 4 and 5 are explained primarily by the fact that in the isotopic method of investigation only P^{32} newly absorbed by plants during the hours elapsed in the experiment is accounted for, while in determining P^{31} the total phosphorus content in the sap and the plant is accounted for.

Only by the introduction of a new isotope can the amount of an element taken up in a short period of investigation be established. But determination of the truly assimilable amounts of the element by plants, based on calculating radioactivity can only be done when the isotopic leveling occurs in the total reacting volume, and when the total element supply at the plant's disposal will become equalized in isotopic composition in every cell of the plant. This process evidently is a protracted one and special investigations are required to establish appropriate periods.

Until then, wherever possible, it would be expedient to utilize data on radioactive and ordinary isotope contents. Such comparisons would permit more reasonable conclusions.

SUMMARY

Corn plants were grown in water cultures at two levels of phosphorus concentration. Later on P^{32} was introduced in the nutritional medium and its uptake by the whole plant as well as the amount of it exuded in the bleeding sap by roots of plants with excised aerial organs were studied.

Plants which were grown before the experiment at a low phosphorus level were found to absorb this element at a higher rate. However in both cases the root system of a whole plant sent much more P^{32} to the aerial organs during 24 hours than was exuded in the sap by the roots of plants deprived of aerial organs Table 2 and 4).

A comparison of this type could not be carried out without aid of the isotopic method and heretofore it has usually been assumed that the sap exuded by the root system approximately reflects the nutritional level of the plants.

In view of the results obtained in the present study one should be cautious in employing the sap method.

Comparison of the amount of P^{32} exuded during 24 hours by plants supplied with P^{32} at the instant of excision of the aerial organs and by plants which were supplied with P^{32} at an earlier period (72 hours) showed that with time the amount of P^{32} in the sap increased, inasmuch as the isotopic composition of phosphorus only gradually leveled out (plants No. 2 and 3 in Table 5).

LITERATURE CITED

[1] A. L. Kursanov and O. N. Kulaeva, Fiziol. Rastenii 4, 332 (1957).*

*See English translation.

[2] O. N. Kulaeva, E. I. Silina and A. L. Kursanov, Fiziol. Rastenii 4, 520 (1957).*

[3] E. I. Ratner, Plant Nutrition and Activity of Their Root Systems. Timiryazev Lecture XVI [in Russian] (Izd. AN SSSR, 1958).

[4] D. A. Sabinin, Physiological Bases of Plant Nutrition [in Russian] (Izd. AN SSSR, 1955).

[5] N. A. Pristupa and A. L. Kursanov, Fiziol. Rastenii 4, 417 (1955).

[6] I. N. Andreeva, Fiziol. Rastenii 4, 533 (1957).*

[7] D. V. Shtrausberg, Labeled Atoms in Investigations of Plant Nutrition and Utilization of Fertilizers [in Russian] (Izd. AN SSSR, 1955).

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* See English translation.

FLOW OF ASSIMILATES TO THE CONDUCTING TISSUE IN RHUBARB (RHEUM RHAPONTICUM L) LEAVES

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With the aid of labeled atoms considerable success has been achieved during the last few years in the study of the translocation of materials in plants. In these studies especially great attention has been given to the transport of assimilates in the phloem. As a result, the direction and rate of translocation of substances has been determined quite exactly and the kind of sugars being transported has been ascertained [1].

During this time however, the transport of noncarbohydrate species (amino acids, organic acids, polyphenols, etc.)has remained almost completely uninvestigated. The question as to the mechanism of this translocation is also controversial, i.e., as to the forces causing the movement of organic materials embedded in the cellular cytoplasm. The solution of these problems is required in future experimental investigations.

A more exact knowledge of the flow of assimilates from the green cells of the leaves into the conducting system could be of great value for clarification of the overall picture of translocation of materials in the phloem, i.e., the very first steps in translocation during which the organic materials of the assimilatory cells are drawn into the realm of the transport mechanism which is active here. Apparently, also, during these early stages,to a certain degree,composition of the materials which are channeled from the leaves into other organs is already determined.

Since this interesting area has still not been subjected to direct study, during 1958 we undertook an investigation in which, by using $C^{14}O_2$, we determined the composition of the photosynthesis products formed in green leaves and at the same time studied the flow of each of the tagged components in the finely branched conducting strands permeating the leaf blade.

METHODS

Rhubarb (Rheum rhaponticum L.) was used in the experiments. The coarse leaves of this plant and the definite net of veins made it suitable for tagging studies. Two months before the study began,two-or three-year-old roots of rhubarb were transplanted into ceramic jars containing soil. Completely mature leaves were taken for for the experiments.

With normal daily illumination of a large part of the leaf and without separating it from the plant, we clamped onto the leaf the two sides of a flat glass chamber(Novitskii's chamber [2]) through which, during the experiments, was cycled a gas mixture containing tagged carbon dioxide. The total concentration of CO_2 in the chamber was 1% the radioactivity 2μ C. In all cases photosynthesisoccurred exactly two minutes in the presence of $C^{14}O_2$.

After removal of the chamber the treated areas were left on the plant for three more minutes in order to provide time for outflow. Then they were cut out and under a lens, with transmitted light and,with fine cutting tools, they were dissected out as: a) mesophyll enclosed between the veins of fourth and fifth order branches, b) fine veins of the fourth and fifth order and, c) the prolongation of these veins into regions which adjoin the area supplied $C^{14}O_2$, i.e., the part of the conducting pathway not directly in contact with the $C^{14}O_2$ which already contains the first portion of the tagged assimilates being transported (figure).

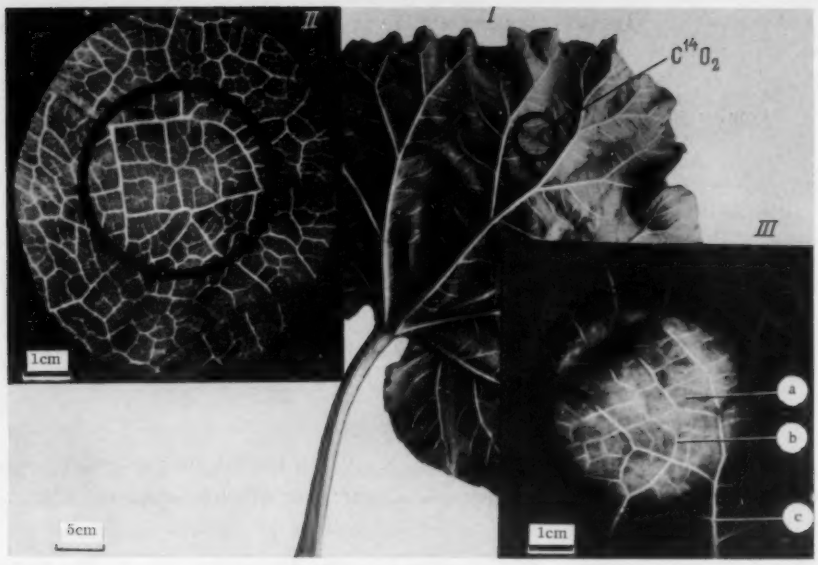

Fig. Experimental arrangement and sampling for analysis: 1) overall view of a leaf and the location of the chamber applied; 2) nature of the venation; 3) radioautograph of the leaf area after exposure to $C^{14}O_2$; a) mesophyll tissue; b) 4th-5th order conducting traces; c) extension of the conducting strand beyond the limits of the chamber.

The dissecting operation consumed another two minutes. Therefore, counting from the beginning of photosynthesis in $C^{14}O_2$ to the end of the dissection into fractions,the experiment took seven minutes.

The dissected samples were immediately fixed in boiling 96% ethanol. After removal of the alcohol in a Vartapetyan apparatus [3] for drying at low temperature,the material was analyzed.

Even with very careful work each of these three fractions was never free of the other fractions. However, fraction (a) consisted chiefly of chlorophyll-rich leaf parenchyma containing, as shown by microscopic examination not more than 10-15% of fine fibrovascular bundles. Fraction (b), on the other hand, consisted chiefly of conducting bundles surrounded by leaf parenchyma cells. The latter part amounted to no more than 20%. Finally, fraction (c), in contrast to fraction (b), contained radioactive materials in only the conducting system and therefore was the best index for determination of the composition of materials conducted from the mesophyll to the phloem.

One portion of the dried material we analyzed for sugars and in the process ascertained the total radioactivity of the acids and amino acids. In this analysis 200-300mg of powdered material was extracted three times with hot 80% alcohol. The alcohol extract, containing the sugars, organic acids, and amino acids was lyophilized and the residue taken up in water.

In order to separate the sugars from the other materials, the water extract was subsequently put through a cation exchanger, Dowex-50, which retained the amino acids, and through an anion exchanger, PE-9, which captured the organic acids. A special test showed that this completely freed the solution of amino acids without loss of sugars. This was particularly important for establishing the radioactivity of the individual components.

The aqueous solution of sugars was vacuum distilled and the residue transferred to 80% ethanol. The solution containing mono-, di- and some oligosaccharides was partitioned by one direction paper chromotography in a butanol-acetic acid-water (4:1:5) solvent. After elution from the paper the individual sugars were quantitatively determined by the anthranone method [4]. In order to determine the radioactivity of the individual sugars, their spots were eluted from the paper with alcohol and after drying in a dessicator were counted with an end-window counter. On the same sample of material during this process we determined the total radioactivity of

TABLE 1

Distribution of Radioactivity in Various Groups of Materials in Zones a, b, c after Two Minutes of Photosynthesis With $C^{14}O_2$ and Three Minutes of Translocation (average of four experiments)

Zone	Total radioactivity of alcohol extract		Radioactivity in groups of materials (% of total)			
	counts/min. · g dry matter, in thousands	% of zone a	sugar	amino acids	organic acids	other materials
a	4460	100	70.0	6.6	6.2	17.2
b	2300	52	72.0	10.5	6.3	11.2
c	70	1.6	73.5	11.5	6.0	9.0

the amino acids after eluting them from the Dowex-50 column with 10% NH_4OH [5]. The total activity of the organic acids was determined by the difference between counts in the solution before and after the anion column, PE-9.

Organic acids and amino acids were determined in a second sample of material. The organic acids were extracted from the dry material with ethyl ether acidified with H_2SO_4 [6]. After evaporation of the ether the residue consisting of organic acids was taken up in warm water and in this form was partitioned by paper chromatography. Two solvents were employed for partition of the acids: 1) butanol - formic acid - water (18:2:9) and 2) ethyl ether - formic acid - water (14:0.2:1.8). For quantitative determination of each of the acids without development, their spots were washed from the paper with water and titrated with .001N NaOH in the presence of a combination indicator consisting of one part of a 0.1% aqueous solution of sodium creosol red and three parts of a 0.1% aqueous solution of thymol blue. The end of the titration was established at pH 8.2. Titration precision with this indicator is 5-10%.

Identification of the individual acids was accomplished with the aid of references. The radioactivity was determined after elution of undeveloped spots with 80% ethanol.

Amino acids were determined in the same sample of material after separation of the organic acids. The dry residue was extracted twice with water at 40-45°; the water extract was put through Dowex-50 and then the amino acids retained on the cation resin were quantitatively eluted with 10% NH_4OH. The mixture of amino acids, freed of ammonium hydroxide and transferred to alcohol, was partitioned by one direction paper chromatography. Partition was accomplished with the solvent butanol - acetic acid - water successively in ratios of 4:1:5 and then 8:3:1. The amino acids were developed with ninhydrin and quantitatively determined on the photoelectric-colorimeter [7].

Identification of the amino acids was accomplished with the aid of references by color development with isatin [8], and in certain cases by rechromatography.

Finally, to determine the radioactivity of the individual amino acids we extracted their undeveloped spots with 80% ethanol and having dried the extracts in the dessicator we counted on an end-window counter.

EXPERIMENTAL SECTION

The experiments showed that in rhubarb, after two minutes of photosynthesis in the presence of $C^{14}O_2$, the radioactivity in zone (a) (mesophyll) is concentrated chiefly in the sugars. Even during these first minutes, however, part of the C^{14} has already entered the amino acids and organic acids of which the former usually somewhat predominate. At the same time, other radioactive products are formed, the exact nature of which is still not clear. In a sample of these latter materials in the assimilatory cells of rhubarb are found about one-sixth of the total amount of $C^{14}O_2$ assimilated. All of this, evidently, indicates the diverse quality of the direct products of photosynthesis [9,10]. In individual plants and under different conditions the distribution of C^{14} among the groups of materials may vary somewhat. However, their fluctuation in general is not great, which permits us to restrict ourselves to average values (table 1).

TABLE 2

Radioactivity of Different Sugars in Zones a, b, and c after Two Minutes of Photosynthesis with $C^{14}O_2$ and Three Minutes of Outflow (per gram dry weight)

Leaf age	Sugars	Zone a		Zone b		Zone c	
		sugar content, mg	radioactivity, counts, imp/min, in thous.	sugar content, mg	radioactivity, counts, imp/min, in thous.	sugar content mg	radioactivity, counts, imp/min, in thous.
Juvenile	Sucrose	23.2	2822	21.3	1816	18.8	55.2
	Glucose	12.4	370	11.9	330	6.7	5.5
	Fructose	5.7	137	7.5	256	4.3	5.2
	Oligosaccharides	1.8	25	—	—	—	—
	% Sucrose	53.8	84.1	52.3	74.5	62.5	83.8
Mature	Sucrose	16.8	2777	11.4	909	11.6	27.3
	Glucose	6.7	198	6.9	85	7.2	3.1
	Fructose	5.7	105	4.7	63	4.1	1.6
	% Sucrose	57.5	90.1	49.9	86.0	50.8	85.1

By comparing the relative amounts of different groups of substances in zones a, b, and c, one can formulate a hypothesis concerning the relative rates of their formation and entry into the conducting system. In particular, it is apparent from Table 1 that 5-7 minutes after the beginning of photosynthesis the radioactivity is found not only in the veins beneath the chamber (zone b), where it could be linked with a direct assimilation of $C^{14}O_2$ by the green cells surrounding the traces, but also in zone (c), that is, in portions of the veins not directly in contact with the $C^{14}O_2$.

During the comparatively short exposures which we used, the radioactivity of zone (c) averaged 1.6% of the mesophyll radioactivity. However, on an absolute basis this is expressed by a quite large number of counts, which permits determination of the radioactivity of individual groups of substances which penetrate into the conducting system.

As Table 1 shows, the radioactivity of the sugars predominated over that of the other substances not only in the mesophyll (zone a) where the sugar was formed during photosynthesis but also in the conducting traces into which they flowed.

In the course of the outflow of materials from zone (a) toward zone (c) there was also observed a tendency for an increase in the radioactivity of the sugars. All of this points out the particularly swift entry of the sugars into the conducting system and their dominating significance in the overall outflow of assimilates. This stimulated us, primarily, to a more detailed study of this group of substances.

Sugars

In Table 2 are presented the results of two experiments which characterize the composition of the sugars in zones a, b, and c in young and mature leaves. Also given is the radioactivity of individual sugars formed during photosynthesis (zone a) and entering into the transport channels (zones b and c).

The data in Table 2 show that sucrose, glucose and fructose are chromatographically found in the leaves of rhubarb. Sometimes, to this is added a quite small group of oligosaccharides which follow sucrose closely on the chromatogram. In general, however, the oligosaccharides are not typical of rhubarb leaves. More characteristic is the ratio between the individual sugars which is maintained quite steady both in the assimilatory and in the conducting tissues. It is expressed in the comparatively small sucrose content (slightly more than half of the total sugars) and in the notable predominance of glucose over fructose.

However, during short-period photosynthesis in an atmosphere containing $C^{14}O_2$ up to 90% of the radioactivity is concentrated in sucrose and only 10-16% goes into the monose portion (see zone a). Probably, this indicates that, in rhubarb as in a majority of other plants, the first free sugar photosynthesis is sucrose. Monoses probably arise secondarily as a result of sucrose inversion. Not excluded, however, is the possibility that the comparatively small percent of sucrose in the composition of the overall sugar reserve in the mesophyll is caused

by a more rapid separation of this disaccharide from the assimilating cells into the conducting system which leads to a preferential accumulation of monoses in the mesophyll.

Actually, analysis of the fibrovascular traces of these same leaves (zone b and c) shows that here also radioactive sucrose strongly predominates over radioactive monoses and that, consequently, the sucrose enters the conducting pathway in more appreciable amounts. This can be judged conclusively from the analysis of the conducting strands outside the boundaries of the chamber (zone c) since in them the radioactivity should be carried by substances emerging into the channels of translocation. These analyses show that during the initial minutes after photosynthesis begins the sucrose fraction constitutes 84-85% of the sugars entering the conducting system.

We still know little about the distribution of sugars in the separate parts of photosynthesizing cells and about the extent of their accessibility and the connection of this with the outflow. However, if we assume that the total of stored sugars determined analytically, corresponds to the amount potentially available for outflow of sugars, we must conclude that, whereas sucrose approaches 54-57% of the total of stored sugars in the zone, in the translocation channels the sucrose content of the mixture is 84-85%. This indicates that sucrose is concentrated in the conduction tissues or, what amounts to the same, the sucrose flows selectively from mesophyll cells into conduction system.

TABLE 3

Radioactivity of Different Acids in Zones a, b, and c after Two Minutes of Photosynthesis with $C^{14}O_2$ and Three Minutes of Outflow (per gram dry weight)

Acids	Amounts by zones, mg			Radioactivity by zones, cpm		
	a	b	c	a	b	c
Oxalic	1.31	1.32	1.96	1370	2110	0
Tartaric(?)	2.76	2.67	2.21	770	1500	0
Citric	1.26	1.33	2.66	3880	1840	610
Malic	2.42	3.68	4.59	6110	5000	1100
Succinic	0.10	0.17	0.11	2070	360	0
Fumaric	0.10	0.14	0.05	2240	660	0
Total	7.95	9.31	11.58	16 440	11 470	1710

Organic Acids

As already observed, after a short photosynthesis in the presence of $C^{14}O_2$ about 6% of the C^{14} assimilated by the leaf appears in the organic acids. (see Table 1).

During this period radioactive acids are found not only in the assimilatory cells (zone a) but also in the conducting system (zone b and c) from which it could be concluded that acids too in some measure participate in the overall outflow of assimilates. More detailed analyses permitted us to establish differences in the behavior of individual acids. The results of one such experiment are presented in Table 3.

It is evident from table 3 that the total content of acids increases from the mesophyll to the veins in rhubarb leaves. This increase occurs chiefly because the zone of conducting tissues (b and c) contains more malic and citric acids, the amount of which, for example, in the experiment cited, about doubled from zone (a) to zone (c). Moreover, in the rhubarb leaves we chromatographically located a rather large spot which corresponded in R_f value to tartaric acid but could consist of several compounds. Also, the leaves always contained an appreciable amount of oxalic acid and a little succinic and fumaric.

That malic and citric acids accumulate in the zone of conducting tissues is probably because they are transported most actively. However, this does not exclude the possibility that these acids originate in the vein tissues secondarily and have no direct relation to transported products. Resolution of this problem might be reached through observation of the movement of tagged acids.

From the right hand part of Table 3 it can be seen that after two minutes of photosynthesis with $C^{14}O_2$ all of the acids in the mesophyll appear tagged. The greatest radioactivity is concentrated in the malic and citric acids in which nearly 60% of the radioactivity in the acids is found. However, fumaric and succinic acids are also tagged very strongly during the first minutes of photosynthesis, although the total amount of them in the leaves is ordinarily not large.

In zone (b), that is during the flow of the assimilates from the mesophyll into the conducting system, the radioactivity of malic and citric acids still remained high here also consisting of a total of 60% of the total radioactivity of the acids in this zone. Here though, the oxalic and tartaric (?) acids were accumulated while the succinic and fumaric acids weakly penetrated from the mesophyll into the conducting zone. However, this

TABLE 4

Radioactivity of Amino Acids in Zones a, b, and c after Two Minutes of Photosynthesis with $C^{14}O_2$ and Three Minutes Outflow (per gram dry weight)

Amino acids	Amount by zones			Radioactivity by zones counts · 10^{-3}/min		
	a	b	c	a	b	c
August experiment						
Aspartic	0.21	0.37	0.06	19.68	2.96	0.14
Serine	0.63	0.55	0.33	76.23	43.15	1.52
Glycine	0.38	0.27	0.17	22.01	16.08	0.49
Glutamic	0.91	0.79	0.63	15.92	9.07	0.64
Threonine	0.49	0.44	0.15	7.15	6.30	1.28
α-alanine	0.42	0.42	0.37	57.08	42.89	0.87
Proline	—	—	—	5.19	5.71	0.25
γ-aminobutyric	0.30	0.20	0.11	6.84	6.67	0.09
Total	3.34	3.04	1.82	210.10	132.83	5.28
September experiment						
Aspartic	0.02	—	—	4.06	1.65	0
Serine	0.34	0.48	0.33	27.59	43.26	0.52
Glycine	0.15	0.26	0.22	10.31	6.22	0.30
Glutamic		0.88	1.00	7.15	4.43	0.21
Threonine	1.18	0.14	0.14		1.29	1.26
α-alanine	0.36	0.37	0.30	34.87	38.28	0.80
Proline	—	—	—	12.70	2.42	0
γ-aminobutyric	0.16	0.17	0.17	3.09	0.86	0.49
Total	2.21	2.30	2.16	99.77	98.41	3.58

regrouping of acids still does not signify a loss of a portion of each of them in their own transport but appears to be a reflection of the more rapid metabolism of the tissues surrounding the fibrovascular bundles.

A true determination of the composition of the acids which flow into the translocation channels before the others could be studied only by the analysis of zone (c). As evident from Table 3 in zone (c), that is in the conducting system, malic acid and a small amount of citric begins to appear first. The other acids, at least during the first five minutes after photosynthesis begins are not detected in the fibrovascular traces. In only one experiment (not presented here) were we able to find in zone (c) slight radioactivity, as well, in fumaric acid.

These data thus lead to the conclusion that the flow of organic acids form the mesophyll into the conducting system, as was true in the flow of sugars, is of a selective character.

Amino Acids

Of an even more graphically selective character in the flow of assimilates into the conducting system is that of the amino acids.

In Table 4 are presented the results of two experiments carried out in August and September of 1958 from which it is apparent that during August the total amount of amino acids in the mesophyll was somewhat greater than during September; in the veins though the difference was insignificant. Typical of all zones of the leaves of rhubarb is the predominance of glutamic acid among the free amino acids and also the relatively great amount of serine and alanine. On the other hand, aspartic acid and in part γ-aminobutyric are present in small amounts.

After two minutes of photosynthesis all amino acids in the mesophyll (zone a) appeared strongly tagged with C^{14}. The total radioactivity of the amino acids in the August experiment was twice as great as in September. However, the nature of the distribution of C^{14} in the individual amino acids in both cases seemed quite similar which indicated the systematic nature of amino acid formation during photosynthesis.

The greatest radioactivity was concentrated in alanine and serine, that is, in amino acids formed from pyruvate and oxypyruvate. Here 62-63% of the total amino acid radioactivity was found. Less radioactive was the group of amino acids formed from α-ketoglutaric acid, that is, glutamic and its derivatives γ-aminobutyric, threonine, and proline. In these was concentrated from 17% (August) to 23% (September) of the amino acid radioactivity. About 10% of the C^{14} was found in glycine which, probably, is linked with the formation of glyoxalic acid during photosynthesis. Finally, from 4% (September) to 9% (August) of the radioactivity in rhubarb during photosynthesis entered aspartic acid which is formed from oxaloacetic acid.

In zone (b), that is in the veins beneath the chamber, the total radioactivity of the amino acids in one experiment was somewhat lower and in the other nearly equal even to the radioactivity in the mesophyll. However a marked redistribution was observed within the same group of amino acids. In the August experiment in zone (b) the amount of radioactive alanine was 25% less than in the mesophyll serine 44% less, but aspartic acid even 86% less. While the radioactivity of γ-aminobutyric acid decreased a total of 2%, the activity of proline increased as much as 10%. The other amino acids likewise behaved differently. As a result, the original proportions of amino acids, made up in the mesophyll during photosynthesis appears to be substantially altered during transfer into the conducting traces (zone b).

Apparently, these changes are not strongly systematic but express only the plant condition during the given period since, for example, in September the regrouping of tagged amino acids in zone (b) assumes a different character than in August.

These differences develop in particular in September when tagged alanine and serine not only do not decrease in zone (b) but even appreciably accumulate in comparison with the mesophyll: alanine 10% and serine 57%. The radioactivity of the other amino acids decreases to various degrees, the radioactivity is especially strongly curtailed in γ-aminobutyric acid (72%) and proline (81%), that is in those amino acids which showed a tendency to accumulate in the August experiment.

The regrouping of amino acid radioactivity in the zone transitional from the assimilatory to the conductive tissues (zone b) still does not characterize the composition of the amino acids which first enter the channels of translocation. The results of an analysis of zone (c) can give an idea of this. As is apparent from Table 4, threonine strongly predominates in the mixture of tagged amino acids which succeed in entering the conducting system after five minutes. This amount constitutes 24% in the first experiment and in the second experiment even 35% of the total amino acid radioactivity while the radioactivity of the threonine component in the mesophyll approaches 3.4% of the total and in zone (b) 1.3-4.7%. Since the total amount of threonine in zone (c) is not large (see the left half of Table 4 it could hardly be hypothesized that the conductive tissues themselves can synthesize it so quickly from other substances. Therefore, the large amount of radioactive threonine in the channels of translocation ought to be considered a direct result of its more rapid flow from the mesophyll into the conduction system.

In addition to threonine, the alanine and serine in zone (c) also exhibit a relatively great radioactivity. Besides, the appreciable efflux of these amino acids into the conducting system also corresponds with the greater amount of them in the assimilatory tissues.

Aspartic acid, and in individual experiments proline or γ-aminobutyric acid, appears to be slightly active. As a result, the relative composition of the amino acids which flow into the conducting system during the initial minutes differs from the composition of the radioactive amino acids of the mesophyll and also from the total amino acids stored in the leaf parenchyma.

We are thus led to the conclusion that within a group of amino acids the flow of individual acids from the mesophyl into the conducting system occurs with differing speeds and furthermore is not always proportional to the concentration of the acid in the mesophyll.

The most typical feature of the efflux of amino acids in a given plant is the rapid partition of threonine into the channels of translocation.

All this leads to the conclusion that the flow of assimilates from the mesophyll into the conducting tissues comes about not as a result of the free diffusion of substances but has a selective character controlled by the metabolic processes.

Just as this work was concluded the two papers by Nelson and Garham [11] appeared in which, with the aid radioactive amino acids deposited on a cut leaf, it was shown that in young soya bean plants translocation of amino acids occurs at various rates and often in different directions. The authors did not have tagged threonine at their disposal; however, they were able to show the great mobility of serine and alanine which is exhibited in definite growth of the plants. The close coincidence of these results with our data affirms the basic conclusion that the flow of individual amino acids and likewise other products of assimilation from the mesophyll into the conduction system in plants is selective.

SUMMARY

Small sections of intact rhubarb leaves were exposed, for 2 minutes, to light in an atmosphere containing $C^{14}O_2$ with the aim of studying the translocation of photosynthates from mesophyll cells to conducting tissues. Three minutes later the sections were divided into the following zones: a) mesophyll with assimilated $C^{14}O_2$, b) veins of fourth-fifth order adjacent to the mesophyll, c) extensions of the same veins not lying in the region of assimilated $C^{14}O_2$ (see figure).

From an analysis of the samples the following conclusions can be drawn.

After 2 minutes of photosynthesis in the presence of $C^{14}O_2$ sugars, amino acids, organic acids and a group of unidentified substances are labeled in the rhubarb leaf. They are formed in the proportion 70:7:6:17.

Even during the first few minutes the assimilates formed in the mesophyll enter the conducting system, sugars and amino acids being the first to enter and organic acids and some other compounds being absorbed at a slower rate (Table 1).

Sucrose is the first compound to be formed in rhubarb leaves during photosynthesis. This disaccharide is also the first to enter the translocation stream and comprises about 84-85% of the total amount of sugars. In the mesophyll and tissues surrounding the conducting path the sucrose content, on the other hand, is 50-60%. This indicates that entrance of sucrose in the conducting tissues is of a selective nature (Table 2).

Malic and citric acids are the organic acids which are especially strongly labeled in photosynthesis. These acids are also the first to enter the translocation stream, their proportion being 2:1. Other acids and especially fumaric, succinic and oxalic, which also become strongly radioactive during photosynthesis (at least during the first few minutes), do not enter the stream (Table 3).

Alanine and serine are the amino acids which are most strongly labeled. However, threonine enters the conducting system with greatest ease, although the fraction it comprises in assimilating cells is not large. Serine and alanine also penetrate the translocation stream with relative ease. On the contrary aspartic acid, proline and sometimes γ-aminobutyric acid only weakly participate in the flow (Table 4).

The results obtained lead one to the conclusion that movement of assimilates from the mesophyll to the conducting tissues cannot be ascribed to free diffusion of the substances but rather should be regarded as a selective process which is controlled by metabolic processes.

LITERATURE CITED

[1] A. L. Kursanov, The Coupling of Physiological Processes in Plants [in Russian], Timiryaz. Lecture XX. (Izd. AN SSSR, 1959) [in press].

[2] Yu. I. Novitskii, Fiziol. Rastenii 3, 574 (1956).

[3] B. B. Vartapetyan, Fiziol. Rastenii 3, 579 (1956).

[4] O. A. Pavlinova, Fiziol. Rastenii 4, 98 (1957).*

[5] O. Samuelson, The Use of Ion Exchange in Analytical Chemistry (IL, 1955) [Russian translation].

[6] M. Ya Shkol'nik, Doklady Akad. Nauk SSSR 90, 5, 847 (1953).

[7] G. N. Zaitseva and A. N. Belonzerskii, Mikrobiologiya 26, 5 (1957).*

[8] A. N. Boyarkin, Fiziol. Rastenii 3, 381 (1956).

* See English translation.

[9] A. A. Nichiporovich, Photosynthesis and the Theory of Obtaining High Yields [in Russian] Timiryaz. Lecture, XV. (Izd. AN SSSR, 1954).

[10] A. Moiz, Fiziol. Rastenii 6, 274 (1959). *

[11] C. Nelson and P. Garham, Canad. J. Bot. 37, 3, 431, 439 (1959).

* See English translation.

THE INTERRELATIONS OF AMINO ACIDS ON THEIR ABSORPTION BY THE ROOTS OF WHEAT

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The question of the methods and mechanism of the absorption of amino acids by plant tissues, particularly by the roots, has not as yet been adequately touched upon by experimental investigations, although the individual works on this question that are available are doubtless of interest. Thus, Arisz and his colleagues [1-3] studied the absorption of asparagine by such specific plant tissues as the delicate hairs of the leaves of sundew (Drosera capensis) and the leaves of Vallisneria. Because of the specificity of the objects selected, the processes of primary absorption and further movement cannot be sharply separated here, a fact also noted by these authors [1]. In addition to this, these authors used concentrations of amino acids which were too high in many experiments. However, in spite of these complications, they were able to show that the features of the absorption of amino acids with the study objects in many ways were correlated with the features of the absorption of nonorganic ions. In particular, the great importance of the supply of oxygen and the sensitivity to the effect of inhibitors were shown here.

On the other hand, however, several differences were also noted along with these normal features. Thus, some inhibitors (potassium cyanide, iodoacetic acid, sodium arsenite) suppressed the absorption both of phosphate and of asparagine, others (penicillin, sodium, arsenate) suppressed only the absorption of asparagine, and the the third (phlorhizin) showed an effect only on the absorption of phosphate. The amount of the maximum absorption from the concentrated solutions did not change when phosphate and asparagine were placed together in the solution.

These facts led Arisz to the conclusion [1] that amino acids and phosphates in the process of absorption and translocation are connected by different constituents of the protoplasm and, therefore, their absorption and accumulation in the tissues proceeds in an unconnected manner. On the other hand, in the case of the absorption of amino acids from paired combinations (glycocoll + alanine, or glycocoll + asparagine), their total absorption did not exceed the absorption of each of them from the pure solution and that, according to the authors, suggests their connection with the same constituents of the protoplasm.

In Webster's work [4], in which the absorption of tracers of the carbon of glutamic acid by hypocotyls of pea and discs of beets and potatoes was studied, the conditions of the experiment were more favorable than in the described experiments of Arisz and his colleagues. The author also showed that the absorption of glutamic acid by the listed objects was subject to the inhibiting of respiration (cyanide, azide, and dinitrophenol) and anaerobinosis.

In the investigations of Ratner and Ukhina [5], the absorption of different amino acids by the roots of corn was followed in sterile cultures by means of the analysis of the sap obtained over a period of 24 hours after the introduction of the appropriate amino acids into the root medium, which was practically devoid of nitrogen. The experiments showed that the studied amino acids were arranged in the series glycocoll > glutamic acid > aspartic acid according to the intensity of the intake of nitrogen into the sap.

Interesting details somewhat closer to the nature of the original effects observed by us were obtained in the work of Birt and Hird [6], in which the absorption of amino acids (principally histidine) by discs of beet was studied. The authors came to the conclusion that the curve of the absorption of histidine over time is similar to the curve for the absorption of nonorganic ions. The beginning period of rapid absorption, which is followed by the second period of slow absorption, can be differentiated on it. The first period, which has a duration of about 30 minutes, was not observed to be susceptible to such inhibitors as dinitrophenol and potassium cyanide. According to the ideas of these authors, this beginning period results in the establishment of a physical equality between the histidine in the external solution on one hand, and on the intercellular fluids and on the adsorbing surface of the cells on the other.

The histidine absorbed in the first period can,to a significant degree,be washed off with water. Phenylalanine, arginine and aspartic acid showed the ability to suppress the accumulation of histidine in the tissues. In the case of a pair of amino acids—histidine + arginine—the suppression is observed in both directions; this brings the authors to the conclusion that the same mechanism is at the base of the absorption of both amino acids. Of particular interest are the facts established in this study of the different absorption by discs of beets of D- and L-isomers of amino acids, which also found support in the work of El-Shishini and Nossier [7] with this same object.

We set for ourselves,in our investigations,the goal of tracing the kinetics of the absorption by the roots of amino acids and their interrelationship in the case of amino acids that were both identical and different in their structure. The experiments were carried out with isolated (excised) wheat roots.

OBJECTS AND METHODS OF INVESTIGATION

Winter wheat 2453 was taken for the experiments. The seeds were shaken for a period of 5 minutes in a 0.5% solution of hydrogen peroxide, after which they were placed in vessels for germination on filter paper moistened with distilled water. Germination was carried out in the dark at a temperature of 26°. After 2 days, the germinated seeds were set out on paraffin gauzed jars in containers with tap water, where they continued to grow for 2 more days at a temperature of 26° in greenhouse conditions and with supplementary artificial light. For one half hour prior to the experiment with the absorption of amino acids, for 20 shoots in each variant with two repetitions (2 × 20), the roots were excised and placed in one-half liter jars containing 90 ml of the buffer solution, Na_2HPO_4 + KH_2PO_4, diluted to 1/10 strength (0.0066 M). Ca was added to the solution in the form of $CaCl_2$ at the rate of 1/20,based on the Hellriegel mixture; the pH of the solution was 5.2.

In the experiments we studied the absorption by the roots of glycocoll and tyrosine, marked by the carbon (C^{14}) in the carboxyl group. These amino acids were taken as representative of the amino acids of the fatty (glycocoll) and aromatic (tyrosine) series and, moreover, they are sharply differentiated by their part in the processes of metabolism, particularly in the roots. As previous experiments carried out with these same marked amino acids have shown [8], glycocoll is easily metabolized in the roots of corn, whereas tyrosine is metabolized only to a very slight degree.

The corresponding quantities of the solutions of the indicated amino acids, brought up to a volume of 10 ml, were added to the experimental jars containing the roots of 20 shoots and 90 ml of water. In this way the normal quantity of the solution was brough up to 100 ml.

We drew out the flow of air through the jars with the help of a water-jet pump during the entire period of absorption. The temperatures in the different experiments varied between 16° and 18°. The concentration of the marked amino acids varied in relation to the goal of the experiment from 0.48 to 7.68 μ-equivalents per 10 ml of solution. The activity of the solutions varied from 2.5 to 40 μC per 100 ml. When adding α-alanine (representing the fatty series) and phenylalanine (aromatic series) to the marked amino acids as competing amino acids, the latter were added to the solution in the quantity 19.2 μ-equivalents, which was 10 times as large as the average of the concentrations taken for the marked amino acids.

At the end of the experimental period, the roots were placed in Buchner funnels where, after drawing off the solution, they were double washed with distilled water and then placed for one half hour in jars containing 100 ml of distilled water. This procedure of washing aided in the elimination of that part of the amino acids which, according to the data of Birt and Hird [6], is physically held by the roots and is easily removed by water. Because the treatment of all variants of the experiment took considerable time, the corresponding forms were examined at the beginning and end of each experiment.

After a half hour of washing, the roots were placed in 5-10 ml of 96% ethyl alcohol to stop the biochemical processes, because further work with these roots was continued only on the following day. In the first experiments we used pure alcohol; however, after this, alcohol containing 1% hydrochloric acid was used. This made the homogenization of the roots and the procurement of average samples for analysis easier. The homogenized mass was increased up to 10 ml after treatment of the roots in the homogenizer; corresponding quantities were taken from this for the measurement of radioactivity. The latter was calculated with the help of a face meter after drying the solution on discs. The corrections for self-absorption were not made because the dry substance was practically similar for all variants.

The experimental data given below show the absorption by the roots of amino acids in μ-equivalents for 20 shoots. These data can be calculated also on dry substance, keeping in mind that the dry weight of the roots of 20 shoots was 35-40 mg.

RESULTS OF THE EXPERIMENT AND DISCUSSION

The first experiments were directed at deciding the question of the path of the absorption of the indicated amino acids over time and in relation to concentration. In order to obtain curves of absorption over time, solutions of marked compounds of glycocoll and tyrosine in the concentration of 1.92 μ-equivalents per 100 ml were taken. The absorption was determined after 1/2, 1, 2, 3 and 5 hours. It appeared that the path of the curves of absorption for these amino acids do not coincide (Fig. 1). The process of absorption for glycocoll proceeded energetically for the first three hours and then slowed down. On the other hand, the absorption for tyrosine proceeded relatively slowly in the first three hours, but sharply increased in the following hours.* This suggests the slower development of the process of absorption of tyrosine over time in comparison to glycocoll.

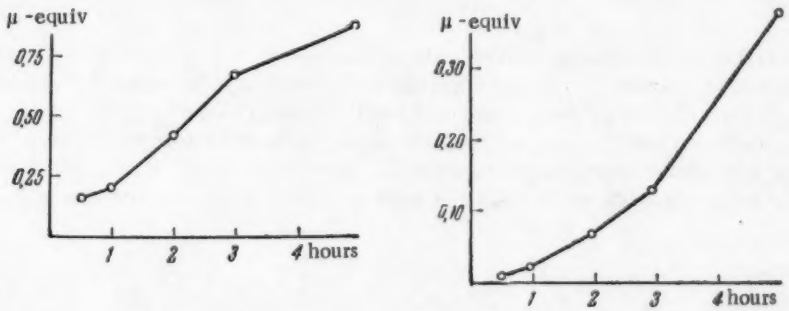

Fig. 1. Curves of the absorption by wheat roots of glycocoll (left) and tyrosine (right) over time.

The reason for these differences can, to some degree, be explained by the differences noted above in the intensity of the involvement of the glycocoll and tyrosine in the process of metabolism which is taking place in the roots [8].

The fact that glycocoll is metabolized in the roots at a significant rate is also suggested by the results of the calculation of the distribution of radioactivity between the fractions extracted and not extracted by alcohol in our experiment with glycocoll. This calculation was carried out in the following manner.

The roots were placed in 96% ethyl alcohol for 15 hours, after which a large part of the alcohol was removed by evaporation in a water bath; the precipitate was decanted from the roots and the roots were carefully washed in succession with alcohol and distilled water. The calculation of the radioactivity of the alcohol soluble fraction showed that it constituted only about 30% of the normal activity of the roots (Fig. 2). The radioactivity of the fraction not extracted by alcohol, which is related to the protein portion, was also determined in several forms, with the courteous participation of T. F. Andreeva. For this the proteins from the homoginizer

*It must be noted that the quantity of roots in these two experiments was not very similar and, therefore, the absolute quantity of the accumulated amino acids cannot be compared here.

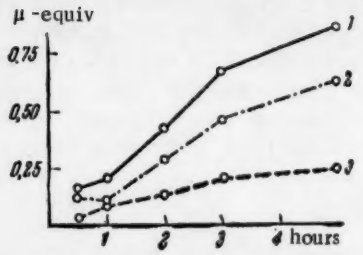

Fig. 2. Distribution of the activity of glycocoll marked with C^{14} in different fractions. 1) Normal activity of the roots; 2) activity of the fraction not extracted by alcohol; 3) activity of the fraction extracted with alcohol.

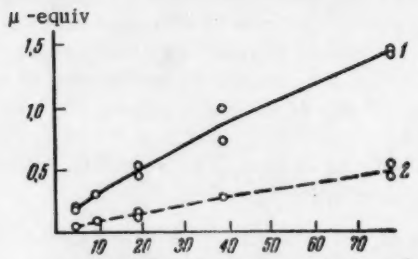

Fig. 3. The effect of the concentration of glycocoll and tyrosine in the solution when absorbed by the roots. 1) Glycocoll; 2) tyrosine

were precipitated by trichloroacetic acid followed by many washings of the precipitate. It appeared that 38% of the normal activity of the roots was found in the protein after only an hour of the experiment; it decreased to 20.3% after 5 hours of the experiment. These figures give an idea only of the order of the size because, as a result of insufficient material, only single determinations were carried out (without parallels).

We note, for comparison, that in the work of Birt and Hird [6] cited above, the L-histidine absorbed after 18 hours and strongly held by the discs of beet was then found in the homogenizer to be practically decanted in the free form.

The second question which we studied in the primary experiments was the path of the absorption of the indicated amino acids in relation to their concentration in the solution. The duration of the experiment was 5 hours. We can see from Fig. 3 that curves were obtained in the range of the indicated concentrations (from 4.8 to 76.8 μ - equivalents per liter) for both amino acids which suggested the adsorption curves. However, the question of the role of adsorption in this process must be left to further investigations, considering the results of the work indicated above [1-4, 6], which suggests a more complicated nature of the absorption of amino acids by plant tissues.

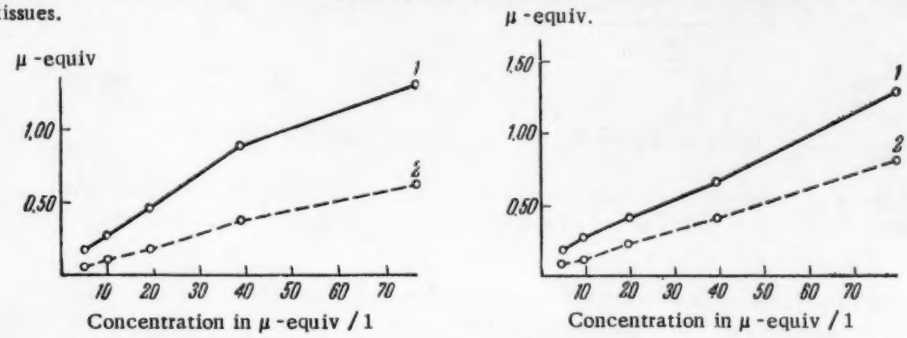

Fig. 4. The effect of alanine (left) and phenylalanine (right) on the absorption of glycocoll. 1) Absorption from a pure solution of glycocoll; 2) absorption from a solution of glycocoll and a competitive amino acid.

In these experiments the absolute sizes of the absorption of tyrosine and glycocoll could be compared. We see from Fig. 3 that the absorption of tyrosine for all concentrations in only about one third the absorption of glycocoll.

After the observations of the primary experiments, the study of the question of the interrelations of the amino acids on their absorption by wheat roots was treated. At the beginning we studied the competitive effect of amino acids similar in structure, for which we added unmarked alanine to the solution of marked glycocoll and unmarked phenylalanine to the solution of marked tyrosine. In the following experiments we studied the competitive effect of amino acids which are not similar in structure (combinations of glycocoll with phenylalanine and tyrosine with alanyl). The duration of the experiments was 2.5 hours in all cases.

TABLE

The Suppression by Alanine and Phenylalanine of the Absorption of Marked Glycocoll and Tyrosine by the Roots of Wheat (decrease in absorption as % of the control)

Concentration in the solution of marked amino acids, μ-equivalents per liter	Decrease in absorption of glycocoll		Decrease in absorption of tyrosine	
	alanine	phenylalanine	alanine	phenylalanine
4.8	66	55	+ 4	29
9.6	47	59	+ 6	7
19.2	59	47	13	14
38.4	57	39	10	5
76.8	52	37	7	25
Average	55	48	4	16

The curves in Fig. 4 clearly show the inhibiting effect both of alanine and phenylalanine on the absorption of marked glycocoll. However, the effect of phenylalanine in this connection is noticeably less than the effect of the alanine: in the first case, the absorption decreased on the average 48% and in the second 55% (Table).

In connection with tyrosine, which, as we saw above, is, in general, significantly less strongly absorbed by the roots than glycocoll, the inhibiting effect of both of the amino acids used was expressed relatively weakly. However, we can see from the data in the table that here glycocoll and phenylalanine changed places: the suppression of the absorption of tyrosine by the roots is more strongly expressed in the case of phenylalanine (the decrease on the average was 16%) than in the case of alanine (the decrease on the average was 4%). In this manner, the similarity or dissimilarity of the chemical structure of the amino acids is an important factor determining their intereffect on absorption by the roots.

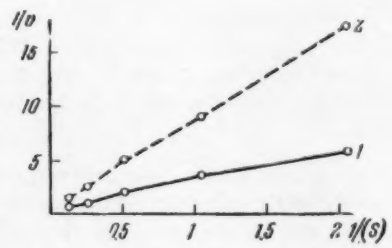

Fig. 5. The effect of alanine on the absorption of glycocoll, expressed in inverses 1/V) inverse of the absorption in μ-equivalents; 1/(S)—inverse of concentration in μ-equivalents per 100 ml. 1) Absorption from the solution of glycocoll; 2) absorption from the solution of glycocoll and alanine.

The data presented can be shown also with the help of a graph of the inverses. This graph for the experiment with glycocoll and alanine is given in Fig. 5. The inverse of the concentration of glycocoll in the external solution is shown on the abscissa and the inverse of its accumulation in the roots is shown on the ordinate. As seen from the graph, we obtain straight lines differing in the steepness of their slope with respect to the abscissa, but having a similar or a close intersection with the ordinate. This answers the question of the clearly expressed competitive inhibition [9]. These same data were also obtained with the combination of glycocoll with phenylalanine.

A similar analysis for tyrosine was puzzling in view of of the weakly expressed inhibition of its accumulation in the roots both of phenylalanine and, in particular, alanine.

The experimental data which we obtained on the absorption of amino acids can be compared with the absorption of nonorganic ions. As we have already indicated, Birt and Hird established that the absorption of amino acids proceeds in two phases. This coincides with the fact that this was also established repeatedly for the absorption of nonorganic ions [10]. It is known that the second phase of absorption connected with the accumulation and the movement of substance in the protoplasm is an aerobic process, responsive to the inhibiting of respiration. This was noted both for the nonorganic and for the amino acids. It was established that the

different amino acids mutually suppressed the absorption of each of them. We were able to show that this suppression has the character of a competitive inhibition and is expressed more strongly for the more competitive amino acid according to their chemical structure. Our results can be to a certain extent connected with the theory of "carrier ions" (Carriers), developed, as is known, to apply to the absorption of nonorganic ions. However, further investigations are needed here, especially in explaining the mutual inhibiting effect in relation to both participating components, (that is, in our case, not only the inhibition of absorption of glycocoll by alanine, but also the reverse). Investigations of a similar nature carried out with nonorganic ions gave interesting and somewhat dissimilar results [11, 12]. A kinetic analysis of the question of the effect of nonorganic ions on the absorption and accumulation of amino acids by the roots is also necessary. We propose that such investigations could give, in particular, valuable material for an appraisal of the correctness of the "theory of carriers" and its applicability to the absorption of different organic and nonorganic substances.

The second of the authors had the honor to work for some time at the K. A. Timiryazev Institute of Plant Physiology at the invitation of the USSR Academy of Science as part of scientific collaboration between the USSR and the Hungarian Peoples' Republic. He wants to express his thanks to all colleagues at the Institute who were of help in his work.

SUMMARY

1. The curves of the absorption over time of the glycocoll and tyrosine marked with C^{14} by the excised roots of wheat are differentiated. In the case of glycocoll, the vigorous absorption taking place in the first three hours is slowed afterwards; in the case of tyrosine, on the other hand, a sharp rise in the curve during the following two-hour period takes place, after the initial three-hour period of slow absorption.

2. In the experiment with a duration of 2.5 hours, the absorption of glycocoll in a broad range of solution concentrations (from 4.8 to 76.8 μ-equivalents per liter) exceeded the absorption of tyrosine by about three times.

A large part of the activity of the absorbed glycocoll by the roots after a short time appeared to be included in compounds not extracted by alcohol, to a significant extent in proteins.

3. The relationship of the absorption of amino acids to their concentration in the solution gives curves both for glycocoll and tyrosine similar to the adsorption curves. However, the question of the role of adsorption in this process needs still further investigation.

4. The absorption by the roots of marked compounds of glycocoll, and to a lesser degree phenylalanine, was suppressed when other unmarked amino acids, alanine and phenylalanine, were added to the solution. The addition of amino acids more similar in structure suppressed absorption more strongly than the addition of less similar amino acids. Therefore, in the case of glycocoll, the inhibiting effect of alanine exceeded the inhibiting effect of phenylalanine, but in the case of tyrosine, it was reversed.

5. The kinetic analysis of the process of absorption of glycocoll in the case of the pairs of glycocoll + alanine and glycocoll + phenylalanine gives a basis for viewing the observed suppression of the absorption of glycocoll under the effect of the other of the named amino acids as a result of the typical competitive inhibition. From this, we can conclude that the absorption of glycocoll, alanine, and phenylalanine is accomplished by the same system in the absorbing apparatus of the roots.

LITERATURE CITED

[1] W. H. Arisz, Proc. Kon. nederl. akad. wet., 45, 1 (1942); Acta Bot. neerl., 2 (1953).

[2] W. H. Arisz and J. Oudman, Proc. Kon. nederl. akad. wet. 40, 431 (1937); 40, 440 (1937); 41, 810 (1938); Chron. Bot. 4, 16 (1938).

[3] J. Oudman, Rec. Trav. Bot. neerl. 33, 351 (1936).

[4] G. C. Webster, Plant Physiol. 29, 382 (1954).

[5] E. I. Ratner, Nutrition of Plants and the Activity of Their Root Systems [in Russian] (Izd. AN SSSR, 1958).

[6] L. M. Birt and J. R. Hird, Biochem. J. 64, 305 (1956).

[7] E. D. H. El-Shishini and M. A. Nosseir, Plant Physiol. 32, 360 (1957).

[8] E. I. Ratner, I. I. Kolosov, S. F. Ukhina, I. N. Dobrokhotova and O. N. Kazuto, Izv. Akad. Nauk SSSR, ser. biol. 6, 64 (1956).

[9] E. Epstein and C. E. Hagen, Plant Physiol. 27, 457 (1952).

[10] D. A. Sabinin, The Physiological Bases of Plant Nutrition [in Russian] (Izd. AN SSSR, 1955).

[11] Z. Boszormenyi and E. Cseh, Nature [in press].

[12] J. F. Sutcliffe, J. Exptl. Bot. 8, 36 (1957).

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APPLICATION OF THE TRACER METHOD IN THE STUDY OF FOLIAR NUTRITION OF PLANTS

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A significant number of works concerned with the large increases in the yields of many agricultural crops as a result of the application of foliar foods have appeared in print in recent years. Yakushkin and Édel'shtein [1-3] propose large-scale production of a new form of food for sugar beets. On the initiative of Uchevatkin and Borodulina [4] in Central Asia, foliar applications of phosphorous foods are used on large areas of cotton.

However, new facts concerned with the low effectiveness of the mineral nutrition of plants through the leaves have now been accumulated in a whole series of cases. For example, investigators concluded on the basis of the work of the All-Union Scientific-Research Institute for Cotton from 1938-1953 on foliar feeding of cotton with phosphorus fertilizers [5] and on the basis of a large number of experiments on foliar feeding of sugar beets carried out in state farms of the horticulture faculty of the Khar'kov Agricultural Institute from 1951-1955 [6] that increases in the yield of cotton or sugar beets that could serve as a basis for the introduction of new agricultural methods in general production were absent. In the experiments with sugar beets to which we referred, 2032 paired comparisons of the sugar of the roots on control and treated plots were made; the average data for these comparisons are as follows: on the control, the sugar of the roots was 18.3% and in the variant with foliar feeding it was also 18.3%. In the field experiments with wheat and sugar beets that we carried out in 1951-1955 [7, 8], foliar feeding in the majority of the cases did not increase the yield and often even led to its decrease.

Many investigators, for example Uchevatkin and Borodulina [4, 9, 10], Yakushkin and Édel'shtein [2-3] and others, giving the theoretical basis of mineral nutrition of plants through the leaves, used the method of tracer atoms. The high effectiveness of foliar phosphorus feeding is shown for this method by the following facts: 1) by observation in the plants of a large quantity of the radioactive isotope P^{32} moving in the form of the corresponding nutrient salt from the solution placed on the surface of the leaves; 2) by the rapid entering of this radioactive phosphorus into the plant organism in the form of organic compounds; 3) by the intensive accumulation of the radioactive phosphorus assimilated by foliar means in the reserve and reproductive organs, which are the most valuable part of the yield.

A large number of radioautographs have been presented by investigators which reflect the observed movement of P^{32} after foliar feeding from the leaves into the roots of beets and carrot, into the fruit of tomato, into the seeds of sunflower, into the boll of cotton, etc. Based on their idea, such radioautographs are an irrefutable documentation showing the high effectiveness of foliar nutrition, reinforced by the "signature" of the radioactive isotope itself.

On the basis of all of those determinations of radioactivity, investigators conclude that phosphorus fertilizers are absorbed and used by the plant in a significantly (many times) larger quantity in foliar feeding than in root feeding.

It is considered necessary to make certain critical observations on this question.

The very vigorous intake of mineral substances from solutions through the sheath of the leaf can be noted, obviously, for most agricultural plants. For example, using P^{32} we repeatedly and invariably observed this for phosphorus in the different periods of vegetation for plants of sugar beet, potato, sunflower, tomato, corn, wheat, barley,

TABLE 1

The Effect of Foliar Phosphorus Application for Summer Wheat, Variety Artemovka, during the Period of Stem Formation on the Concentration of Normal and Radioactive Phosphorus in the Aerial Organs in the Period of Spike Formation (data for 10 plants)

Experiment number	Normal phosporous, mg		Radioactivity phosphorous in treated plants	
	control plants	treated plants	impulses/min, in thousands	mg of P_2O_5 corresponding to the given radioactivity
1	121	129	69.8	24
2	138	130	79.5	27
3	146	152	90.7	31
4	184	179	83.8	29
5	177	191	94.3	32
6	208	213	80.0	27

oats, and others. Knowing the relationship between the radioactive and stable isotopes in the nutrient solution, that is, knowing the coefficient of the isotope separates, we can, of course, also calculate the absolute quantity of the nutrient element moving into the plants. However, this quantity cannot be an indicator for us to the extent that it changes its concentration in the plant organism. Researchers usually overestimate the intensity of assimilation of nutrient substances through the sheath of the leaf and do not consider changes in the root nutrition taking place after foliar feeding. As a number of authors also noted, P^{32} always entered intensively from the nutrient solution through the leaf sheaths in our experiments, but the absolute quantity of phosphorus and other nutrient substances over several days after feeding often would be even less in the aerial organs of the plants (experiments with summer wheat) than in the control, single-age plants, and this usually led to the further decrease in the yield [7].

A direct comparison for individual cases of the concentration of normal and radioactive phosphorus in the aerial organs of summer wheat that was fed through the leaves with a 3% solution of K_2HPO_4 with a tracer of P^{32} in the quantity 0.5 μ/ml is given in Table 1.

Ten plants were grown in each vegetation container on separate soils. The foliar feeding was carried out in the period of stem formation. For a more complete assimilation of the nutrient substances on the surface of the leaves a light dew was applied in the following days to the plants with the help of a sprayer.

Plants were taken for analysis in the period of spike formation (14 days after treatment), whereupon the fresh samples were first of all washed with distilled water. After wetting of the ash, the normal phosphorus was measured on the photoelectrocolorimeter and the radioactive phosphorus on a type B apparatus. Analysis of the normal phosphorus was also made for the single-aged untreated plants (from control containers). Proceeding from the coefficient of the isotope separation, wecalculated thatpart of the normal phosphorus in the aerial organs of the treated plants was there as a result of the foliar feeding and must, therefore, be in excess of the control if root nutrition for the treated plants is unchanged. However, direct determination of the normal phosphorous in the aerial organs shows the lack of grounds for these indirect calculations (Table 1).

The concentration of nutrient substances in the plant as a result of foliar feeding can be noticeably increased only in the case where it does not decrease their intake through the root system in the following time period, that is, if foliar feeding will not oppose root nutrition, but supplement and even increase it.

Observation in the reserve and reproductive organs of large quantities of radioactive phosphorus entering into the plant by foliar means from the nutrient solution, also does not show an increase in the absolute quantity of phosphorus in these organs. In the indicated experiments (Table 2) the plants of summer wheat in the phase of spike formation were fed through the leaves with a nutrient solution "P" or "NPK", marked with the addition of the radioactive isotope P^{32}. This made it possible to compare the absolute concentration of phosphorus and the concentration of P^{32} in the grain.

TABLE 2

The Effect of Foliar Feeding of Summer Wheat, Variety Antemovka, on the Yield of Grain and the Concentration in the Grain of Normal and Radioactive Phosphorus (data given for 10 plants)

Background of soil moisture	Soil moisture as % of capacity	Feeding variants		Main stem			Lateral stems		
		time of feeding, period of development	fertilizer	weight of grain, g	P_2O_5, mg	P^{32} 1000 impulses/min	weight of grain, g	P_2O_5, mg	P^{32} 1000 impulses/min
$N_1P_1K_1$	70	control	—	13.17	171	—	3.15	38	—
		spike formation	P	12.38	156	12.3	4.53	55	9.2
$N_3P_1K_3$	70	control	—	11.89	121	—	9.41	105	—
		stem formation + spike formation	(P+P) +P	11.23	121	11.4	9.07	102	10.2
$N_1P_1K_1$	30	control	—	8.57	77	—	—	—	—
		spike formation	NPK	7.57	67	8.9	—	—	—

Note: On the background of $N_3P_1K_3$, the marked nutrient solution was used only with the third feeding. In Tables 2 and 3, the symbols of the nutrient element with the figure 1 or 3 means one or three doses of the corresponding fertilizers, applied in the soil (a degraded chernozem). With one dose of fertilizer in each container (10 kg of soil, 10 plants) were applied 2 g NH_4NO_3, 2 g superphosphate, 1 g KC_1.

We see from Table 2 that P^{32} was found in the grain in large quantities even when feeding decreased the normal concentration of phosphorus in it in comparison with the control plants.

By the addition of the radioactive isotope, we have in mind not only the normal phosphorus of the nutrient solution, but by introducing it into the leaves, we have in view also the phosphorus of the plant as a result of the isotope exchange. At the time of grain ripening, a vigorous flow of phosphorus compounds from the leaves into the spike takes place; discovering P^{32} in the spike, we thus established the qualitative side of the process taking place in plants, but we cannot in the given case judge its quantitative side (there was one coefficient of isotope separation in the nutrient solution, but after penetration of the phosphorus into the leaves, the coefficient was

TABLE 3

The Effect of Foliar Feeding of Summer Wheat, Variety Artemovka, on the Yield Grain and the Concentration of Ordinary Nitrogen and Phosphorus in the Grain (absolute quantity given as calculated for 10 plants)

Background of soil nutrition	Soil moisture as % of capacity	Feeding variants		Weight of grain, g	N		P_2O_5	
		time of application by period of development	fertilizer		%	mg	%	mg
$N_1P_1K_1$	70	control	—	16.32	3.07	501	1.28	209
		stem formation	N	18.10	3.12	565	1.32	239
			P	17.39	2.81	489	1.24	216
			NPK	19.58	3.07	601	1.32	258
		spike formation	N	17.46	3.11	543	1.28	223
			P	16.91	2.96	501	1.25	211
			NPK	17.12	3.12	534	1.30	223
$N_1P_1K_1$	30	control	—	8.57	3.61	309	0.90	77
		spike formation	NPK	7.57	3.66	277	0.89	67
$N_3P_1K_3$	70	control	—	21.30	3.53	752	1.06	226
		stem formation + spike formation	(P+P) + P	20.30	3.35	680	1.10	223
$N_1P_3K_3$	70	control	—	20.35	2.70	550	1.12	228
		stem formation + spike formation	(N+N) +N	26.84	2.98	800	1.30	349

different because P^{32} already marks at this time a somewhat unknown cumulative quantity of normal phosphorus entering from the solution and being in the leaf). The data given in Table 2 suggest that the intake of P^{32} into the spike will also be noticed in the case where the phosphorus feeding to a significant degree retarded the flow from the leaves and other organs of mineral and organic substances, in particular phosphorus compounds (however, it does not stop it completely).

O. F. Tueva [11] shows that the immediate saturation of the leaf with phosphorus through its sheath does not create conditions for strengthening the flow of this element from it and that the organs in the plants into which phosphorus moves normally consist of tissues with a significantly higher concentration of this element relative to the tissues from which the flow takes place; for the flow and distribution of phosphorus into plants, the character of the tissues in relation to their activity and the intensity and direction of metabolism has importance. One of the factors determining the movement of phosphorus in the direction of the intensively growing tissues, the generative organs, is the constant consumption of its compounds in the process of the synthesis of the protein molecules. O. F. Tueva considers that, obviously, the effect of nitrogen nutrition on the movement of phosphorus in the plant is also connected with this.

In our experiments an agreement of the changes in the accumulation of nitrogen and phosphorus in the grain under the effect of foliar feeding (both nitrogen and phosphorus) was recorded. The change in the concentration of ordinary phosphorus in the grain was usually accompanied approximately by a similar change in the concentration of ordinary nitrogen based on the size of the relative change. Using the data in Table 3, we can calculate that the ordinary concentration of N and P_2O_5 in the grain relative to the control, which is taken as 100% for the different variants of feeding against the background of $N_1P_1K_1$, are respectively: feeding in the period of stem formation with urea—113% and 114%, with superphosphate—98% and 103%, feeding with NPK—120% and 123%; feeding in the period of spike formation with urea—108% and 107%, with superphosphate—100% and 101%, feeding with NPK—107 and 107% (70% soil moisture), 90% and 87% (30% soil moisture); against a background of $N_3P_1K_3$, feeding with superphosphate in the periods of stem formation and spike formation—90% and 99%, against the background of $N_1P_3K_3$, feeding with urea on the same dates—145% and 153%.

In one case three phosphorus foliar applications did not increase the concentration in the grain either of phosphorus or of nitrogen, and in another case three nitrogen applications reduced the concentration both of nitrogen and of phosphorus in the grain approximately 1 1/2 times. Here is a graphic example of the inapplicability for living organisms of the rule of effective mass on which categorical conclusions on the high effectiveness of foliar applications are also based (as is well known, our scientists established the inapplicability of this rule for biological objects long ago). The action of foliar feeding on metabolism is significantly more complicated than a simple mechanical increase in the nutrient substances demanded by the plants. We must consider that excessive mineral nutrients can be injurious not only through the leaves, but also through the roots [12].

The first investigations with tracer atoms established the high mobility of phosphorus in plants and the intensive renewal of organic phosphorus compounds [13]. This is why we observed P^{32} entering very rapidly through the leaf sheaths in the composition of these compounds; the establishment of this fact still does not suggest by itself an increase in the quantity of organic phosphorus compounds or the positive effect of foliar phosphorus feeding on their synthesis. It is obvious that P^{32} can be observed in the composition of organic phosphorus compounds also in the case where feeding with phosphorus even strengthened their decomposition (and also when the processes of synthesis take place, although they are weak). When using the tracer atom method, one must consider the simultaneous presence of two opposite sides of metabolism in the organism (synthesis and decomposition, the movement of some compounds from the aerial organs into the root system, the movement in an opposite direction of other compounds of this same element, etc.).

We are not rejecting the significance of the experimental data which investigators obtained in the study of foliar nutrition of plants by the method of tracer atoms, although we consider that it is necessary to review, critically, many of the conclusions from these facts. A deficiency in the balance of normal (and not only radioactive) phosphorus leads to erroneous conclusions. By one-sidedly using the indicators of radioactivity, we are at the same time ignoring those changes in the metabolism of the plant organism that are a result of foliar nutrition. It must be shown that categorical conclusions on the high effectiveness of foliar feeding of plants made on the basis of investigations with tracer atoms can lead practical agriculture into error. A deeper study into those internal and external factors of plant life that determines the effectiveness of this technical agriculture method is necessary.

It must be understood that all that has been said is directed not against the new methods of investigation in plant physiology, but, on the other hand, towards their effective use by taking into account the features of each method and the rules of the living organism.

SUMMARY

In experiments with summer wheat, facts were obtained that indicated that the discovery in the vegetative organs and grain of plants of large quantities of radioactive phosphorus applied by foliar means from a marked nutrient solution cannot be an indicator of the increase of ordinary phosphorus in the aerial organs, and in particular in the grain, relative to the plants not fed through the leaves. In the case given, one must consider that foliar feeding, particularly with phosphorus, changes (in certain cases, negatively) the intake of phosphorus and other elements of mineral nutrition into the aerial organs of plants through the root system, and also changes the intake of their compounds from the vegetative organs into the reproductive organs.

LITERATURE CITED

[1] I. V. Yakushkin and M.M. Édel'shtein, Foliar Feeding of Sugar Beets [in Russian] (Sel'khozgiz, 1953).

[2] I. V. Yakushkin and M.M. Édel'shtein, Foliar Feeding of Agricultural Crops [in Russian] (Izd. "Znanie", 1955).

[3] I. V. Yakushkin and M.M. Édel'shtein, Collection. Foliar Feeding of Agricultural Plants, edited by I. V. Yakushkin and I. S. Varunyants [in Russian] (Sel'khozgiz, 1955).

[4] F. I. Uchevatkin and A. A. Borodulina, Collection. Foliar Feeding of Agricultural Plants, edited by I. V. Yakushkin and I. S. Varunyants [in Russian] (Sel'khozgiz, 1955).

[5] B. P. Machigin, Collection. Foliar Feeding of Agricultural Plants, edited by I. V. Yakushkin and I. S. Varunyants [in Russian] (Sel'khozgiz, 1955).

[6] G. V. Pilipets, Sakharnaya Svekla, No. 6 (1956).

[7] N. I. Shereverya, The Interconnection of Mineral Nutrition of Plants through the Leaves and Roots. Dissertation [in Russian] (Khar'kov, 1956).

[8] N. I. Shereverya, Sakharnaya Svekla, No. 6 (1957).

[9] F. I. Uchevatkin, A. A. Borodulina, V. I. Dulova and N. V. Vostrilova, Doklady Akad. Nauk UzSSR, No. 8 (1953).

[10] F. I. Uchevatkin and A. A. Borodulina, Collection. Tracer Atoms in Investigations of Plant Nutrition and the Application of Fertilizers [in Russian] (Izd. AN SSSR, 1955).

[11] O. F. Tueva, Collection in Memory of Academician D. N. Pryanishnikov [in Russian] (Izd. AN SSSR, 1950).

[12] N. S. Avdonin, Feeding Agricultural Plants [in Russian] (Sel'khozgiz, 1954).

[13] V. M. Klechkovskii, The Method of Tracer Atoms in Biology, edited by A. M. Kuzin [in Russian] (Izd. MGU, 1955).

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THE EFFECT ON PLANTS OF SOME LITTLE STUDIED MICROELEMENTS

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The normal life of plants and the high yields of all agricultural crops are invariably connected with the conditions of the nutrition for various chemical substances of the organisms. Apparently, every organism requires all or nearly all of the chemical elements, including also the microelements [1].

However, from the tens of different microelements located in all periods and groups of the D. I. Mendeleev periodic system, only a limited number of compounds, basically Mn, Cu, B, Zu, and Mo, are used for research and commercial purposes at the present time. Unintentionally, questions arise: what influences the choice of new microelements, what would be the connection between their position in the periodic system, the structure of atoms and their characteristics, and also what effect could they show on the development of plants?

In our opinion, the different effect of microelements on the different processes going on in all organism can be explained by the chemical characteristics of the corresponding chemical compounds. To a significant degree, the microelements belong to the group d-elements of the D. I. Mendeleev periodic system, that is, those for which the quantitative number of the last added electron is d.

The simple ions with an incomplete external ring with 8 to 18 electrons show an active deforming effect on the electron covering of the components. With an interaction of the ion with a molecule of organic substance, an important role is played by the nature of the ion itself formed by the microelement,, which is formed also by the configuration and, consequently, by the atomic number of the element. It is obvious that the microelements (in the simplest case in the form of ions) and the organic molecules of the tissue show a mutual polarizing action [2].

With polarization, the size of the dipole originating in the ion under the effect of the external electric pole is the immediate extent of the deformation of the ion formed by some microelement. This size depends both on the size of the dislocation of the separate electron orbits of the external energy level and on the number of these levels. Therefore, it must be expected that with stable, equal conditions, the ions with the incomplete outer energy level (for example, Mn^{2+}, Co^{2+}, etc.) are deformed more strongly than the ions with eight electrons in the outer energy level (for example, Na^{1+}, Al^{3+}, etc.). Although to a large extent the ions with an 18-electron outer layer can have a polarizing action (for example, Zn^{2+}, Cd^{2+}, etc.), in a number of cases the incomplete energy level plays a still larger role.

The combination of a strong polarizing action with a relatively light natural deformation is usually characteristic for lightly charged cations with an 18-electron outer layer. The deformation of these cations in one or the same subgroup of the periodic system grows from top to bottom and in this direction the supplementary polarizing effect rapidly increases. Therefore, in spite of the increase in the radius of the ion, the total polarizing effect can be noticeably increased. It is obvious that the more the deformation is interacting with the cation of the anion, the stronger is the polarizing effect.

Recently a similar effect of different cations on the plant organism has been noticed repeatedly in hundreds of works devoted to the different microelements; however, no explanation has been given by these experimental facts. Of course, the electron structure of the ion, the degree of polarization and polarity, and also the sizes of the ion radii show a great effect on the character of the action of the microelement. However, on the

basis only of these characteristics, one can get only an approximate idea of the effect of the given cation on the plant (or animal) object. Such opinions would have the greatest probability in the case where the similar characteristics of the ions belonging to the same subgroup of the D. I. Mendeleev system are present.

The method of analogy, as widely used by the creation of the periodic rule, can also be used for explaining the internally similar effect of microelements in one subgroup. This can be based on the idea that if the ion of a certain chemical element in some manner acts on the organism, then by far the most probable is the same action for its electron analog rather than that for the ions of elements of other groups. Thus, in the secondary subgroup of group II, Zn, Cd, and Hg possess similar structures for the outer electron rings and a similar number of electrons (18 and 2) on the two last energy levels, differing only in their structure on one ring with, respectively, 18, 18 and 32 electrons (atomic number 30, 48 and 80). As is well known, compounds of Zn^{2+}, Cd^{2+}, and Hg^{2+} can appear as different components in the different processes of life, for which the degree of toxicity [3] increases with the atomic number, and which makes it possible to have an approximate idea not only of the necessary doses of the microelement, but also of the character of the effect of the corresponding cations.

The compounds of Zn^{2+} are successfully used as microelements for many plant and animal organisms, but the more toxic compounds of Cd^{2+} practically have not been used up to the present time in agronomy [4-6]; a large literature has accumulated for the most toxic of these, the compounds of Hg^{2+}, and its corresponding toxic effect on plants and animals [3], although the biochemical role of this microelement has not been quite cleared up. Speaking in a broad sense, we can always observe that the substance which in small quantities shows a positive, favorable effect, shows a negative toxic effect in large quantities.

The manifestation of the excellent rule of dialectical materialism, a transfer of quantity into quality with a change in the dose or concentration of substances, is observed at each step. Thus, compounds of Cd^{2+} or Hg^{2+} can in small quantities have a positive effect, but in large quantities, a negarive effect. This applies to all macro- and microelements.

The radii of the cations formed by microelements of the secondary subgroup of group II of the periodic system and the significance of the oxidizing-reducing potentials (Eh) of the leaves when these cations are used is as follows:

Cations	Zn^{2+}	Cd^{2+}	Hg
Atomic number of the elements	30	48	80
Configuration of the electrons on the energetic level	2, 8, 18	2, 8, 18, 18	2, 8, 18, 32, 18
Radius of the cation, in A	0.83	1.03	1.12
Eh, of the leaves, in volts	0.610	0.575	0.553

The polarizing characteristic of the cation is rapidly changed with changes in the size of the ion radius. As we can see from the given indicators in the subgrouping being examined, the radius of the cations increases and, therefore, can change the character of the effect on the plant component. In our experiments, the action of these microelements on grape was studied according to the method described above [7], during which the most important indicator of the system, the oxidizing-reducing potential (Eh), was systematically changed [8]. In order to exclude the polarizing effect of the anion, in our experiments dealing with the foliar feeding of grape, we use only sulfates in doses decreasing with the size of the atomic number: $ZnSO_4 - 5 \cdot 10^{-3}$ g; $CdSO_4 - 5 \cdot 10^{-5}$ g; $HgSO_4 - 5 \cdot 10^{-6}$ g (on the grape bush).

The experimental data that were obtained supported the above considerations on the changes connected wih with the structure and polarizing effect of the microelement. In the series, Zn-Cd-Hg, a decrease takes place in the importance of the Eh, the predetermined intensity of the oxidizing-reducing ability of the given system, and therefore it is possible to predict in which direction the oxidizing-reducing process will move.

The less the importance of Eh of the system having the function of oxidizing and reducing, the greater the degree of the increase in the reducing capacity of the system. Because of the increase in the ease of loss of electrons, the relation of these forms is changed [9] and, in particular, leads to a greater possibility of the reducing of ions of oxygen up to the free condition. Some accumulation of O_2 in the cells can lead to a change in the course of the most important biochemical processes in plants. Thus, this explains the repeatedly observed increase

in our experiments with compounds of Zn^{2+}, Cd^{2+}, and Hg^{2+} in the activity of such oxidizing-reducing enzymes as ascorbinoxydase, polyphenoloxydase, and peroxydase, and also the sharp growth in the activity of catalase, which is a catalyst in the oxidizing-reducing reaction: $2H_2O_2 \rightarrow 2H_2O + O_2$, etc. As Shkol'nik and Abdurashitov [10] showed, there is an immediate connection between the activation of the oxidizing-reducing processes and changes in the character of the carbohydrate metabolism under the effect of the microelements. This extremely important rule is supported entirely by our experiments: the microelements of Zn, Cd, and Hg, while acting very specifically, at the same time similarly aid in the accumulation, for example, of starch in the grain of corn, sucrose in the leaves, and fructose in the grapes, etc.

All of the noted rules in relation to the subgroup Zn can be directed also, for example, to subgroup Cu. Up to this time, only compounds of Cu have been widely used as microelements, but compounds of Ag and Au only very insignificantly. On the basis of the method of analogy, there is undoubtedly a possibility to judge not only the optimal dosage of these microelements, but also the character of their effect.

Among the microelements of significant interest are the elements of the fourth large period of the D. I. Mendeleev system with atomic numbers from 21 to 29—Sc. Ti, V, Cr, Mn, Fe, Co, Ni, and Cu. All atoms of these elements can lose electrons not only from the last energy level, but also from the incomplete next-to-last level, because there is a gradual growth observed in the number of electrons from 8 to 18 (Zn with an atomic number of 30). Therefore, the indicated elements show a different positive valence, especially in complex and internally complex compounds, being in this manner the metal components of the different complex organic substances playing an important role in the life processes.

The radii of the cations with a charge of + 2, formed by microelements with atomic numbers of 25, 26, 27 and 28 and the significance of the oxidizing-reducing potentials (Eh) of the leaves when using these cations are as follows:

Cations	Mn^{2+}	Fe^{2+}	Co^{2+}	Ni^{2+}
Atomic number of the elements	25	26	27	28
Configuration of the electrons on the energetic level	2, 18, 13	2, 8, 14	2, 8, 15	2, 8, 16
Radii of the cations, in A	0.91	0.83	0.82	0.78
Eh of leaves, in volts	0.565	0.545	0.547	0.541

From the described series of microelements, only compounds of Mn and Cu are in relatively broad use [11] and to a certain degree the somewhat costly special compounds of Fe. There are few and fragmented pieces of information on the effect of the remaining microelements on plant organisms. In our experiments in recent years, we studied [1, 7, 12, 13] the action on plant organisms of all (except Sc) of the described components of elements with atomic numbers from 22 to 29.

Only the microelements with atomic numbers of 25 to 28, used in the form of cations with a charge of +2, are compared, considering the great effect of the valence of the ion in the indicators given above. As in the previous case, only the effect of sulfates on grape was studied. From the data obtained, we see that in the series Mn^{2+} - Ni^{2+} the radius of the cation is not changed significantly and also the significance of the Eh is little changed. Actually, the experiments showed the similar analogous positive effect of the compounds of all of these elements on the oxidizing-reducing processes in plants, carbohydrate metabolism, etc.

All of the observed chemical elements theoretically can enter into the composition, for example, of the different enzymes, now and then sharply changing their form in comparison to that with which they first take part in some reaction [14]. Considering that almost all of the now-known conversions of substances are accomplished with the help of enzymes, the fact that the latter series of microelements enter into the compositions is very interesting. [3, 9, 11, 15-20]. Many enzymes represent metal-organic compounds or metalloproteins. Thus, Cu is included in the composition of the oxidizing-reducing enzymes: as carbinoxydase, polyphenoloxydase, laccase; Mn in the composition of arginase; Mo in the composition of xanthinoxydase and aldehydeoxydase; Zn in the the respiratory enzyme of carboanhydrase; Cr in the proteolytic enzyme of trypsin, etc. Also interesting is the recent discovery that the extremely important, cobalt-containing, vitamin B_{12} contains 4.5% Co. A number of

facts of the entrance of different microelements into the composition of the most important substances on which the processes of life are dependent, literally increases sharply every year.

Attention is turned to the presence of an almost constant metallic character (with the rare exception, for example, of compounds of B, which play a specific role) for the overwhelming majority of microelements, which in a free condition do not incorporate electrons, but only lose them. This is related not only to the typical metals of the first group of the periodic system, but also to all other groups down to VIII.

In the composition of the indicated enzymes, vitamins, etc., microelements most often form internally complex compounds, which emerge expressly in the role of metals. The ions of nonmetals, the donors of a coordinating bond, must have at least one free pair of electrons that they can give off to ions of metals. In accordance with this, N, S, and O, but not H and C, can be ion donors.

The ions of metals, playing the role of electron acceptors, must have an unfinished electron ring. The greatest tendency for the formation of internally complex compounds is shown by the ions of metals with two incomplete internal energy levels that is:

Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu
I	Zr	Nb	Mo	-	Ru	Rh	Pd	Ag
La-Lu	Hf	Ta	W	Re	Os	Ir	Pt	Au
Ac	Th	Pa	U					

For ions of other metals, the tendency toward the formation of internally complex compounds is significantly less, but for ions with a completed energy level, only a weak bond is possible. Therefore, while not stopping with the details of this question, it can be said that the idea of the necessity of research on metal components of the products of plant activity is mainly in the indicated direction.

It is not difficult to notice that all of the described microelements can lose electrons from the two outer energy levels and form ions of a different charge, for which there will always be a positive valence. This also predetermines the action of the corresponding microelements in the various oxidizing-reducing processes. As an example, if compounds of Mn^{2+} or Mn^{7+} (say $MnSO_4$, $KMnO_4$) are used, then different transfers are possible: $Mn^{2+} \rightleftharpoons Mn^{3+} \rightleftharpoons Mn^{4+} \rightleftharpoons Mn^{6+} \rightleftharpoons Mn^{7+}$, connected with the specific changes in the E_H of the system. Similarly, when simple compounds of Co are used for which the valence is characteristically + 2, their transfer in the organism is into complicated, complex compounds [21], for which a valence of + 3 is more stable. The transfer of $Co^{2+} \rightleftharpoons Co^{3+}$ is also accompanied by a change in the E_H of the system, as in the case of $Ni^{2+} \rightleftharpoons Ni^{3+}$ and in a number of others.

In our experiments [22] with the microelement Cr, the application of Cr^{3+} in the form of $Cr_2(SO_4)_3$ shows a very favorable effect on the development of grape and several other crops. The possibility of a transfer of $Cr^{3+} \rightleftharpoons Cr^{6+}$ again determined the change in Eh and the action of the microelement in certain oxidizing-reducing reactions, on which the activity of the organism can depend. Actually, from compounds of the elements of the secondary subgroup of group VI of the periodic system, Cr, Mo, W, U, at the present time only compounds of Mo have begun to be widely used in agronomy. Now it is also the turn of other microelements.

All that has been said is connected with the literally unlimited possibilities of involving almost all of the chemical elements of the D. I. Mendeleev periodic system in the circle of the studied microelements.

It is obvious that not only the distribution of electrons on the energy rings and the radii of the ions, but also the total potential for ionization, the energy of ionization, the meaning of the pH of the system and a number of other factors are of great importance [23-25]. However, in the end, the basic determinant is the atomic number of the element. These or other microelements can even change the colloidal-chemical composition of the plasma and direct the activity of the enzyme apparatus of the plants both in the direction of synthesis and in the direction of hydrolysis of certain chemical compounds [26].

One must always consider not the isolated effect of one microelement, but rather the interaction of many chemical compounds. The presence of only one microelement with the absence of others often can be the reason for the cessation of one or another process. From this, the necessity for the presence in the reaction of a certain complex assembly of macro- and microelements with the corresponding interenergy action is clear.

In biochemical processes the role of microelements by no means leads only to a single acceleration of the spontaneously proceeding chemical reactions. In relation to the conditions of application, dosage, the nature of the microelement itself, and a number of other factors, these compounds take on the action of both catalysts predetermining one or another process and of activators or inhibitors of various reactions taking place in the organisms.

We stopped only for a very short time on the small part of our experiments on the study of the effect on plants of such little studied microelements as Cd, Hg, Ti, V, Cr, Co, and Ni. Not having the opportunity to make any sort of comparison or generalization here, we only point out that in the majority of cases, along with the specific action of the cation, a similar consistent effect of the microelements is observed.

The application of $Cr_2(SO_4)_3$ on a southern chernozem with a slightly alkaline reaction (pH = 7.2-7.3) in the quantity 0.005-0.01 g of salt per bush of tomato or eggplant prior to flowering of the plants accelerated flowering and increased the fruitfulness of the vegetables. Thus, the number of flowering plants was six times as high and the first harvest of the eggplants twice as large as that of the control plots.

Some poor, but also some good, results were obtained in similar cases using $CoSO_4$ and $NiSO_4$ in the quantity 0.01-0.1 g per bush. It is curious that the simultaneous application of $CoSO_4$ and $ZnSO_4$ was especially effective; for example, the yield of eggplants increased from 65 to 102 centners/hectare.

The preplanting treatment of seeds of different agricultural crops [27] in solutions containing the salts Zn^{2+}, Cd^{2+}, Cr^{3+}, Mn^{2+}, Co^{2+} and Ni^{2+} showed a favorable effect. We will dwell only on the use of compounds of Co^{2+} for corn. Soaking the seeds for a period of 24 hours in 0.001-0.01% solutions of $CoSO_4$ increased the yield of corn according to the data from several collective farms from 8 to 38%.

It is important that here the chemical composition of the grain is changed in proportion to the acceleration of the transformation of sugars into starch, and this leads to the more rapid ripening of the corn. On the attainment of a technical ripeness in the corn grain grown from seeds treated with water (control), the quantity of ordinary sugar calculated as sucrose was 5.02%, and the quantity of starch was 64.4% (sum of the carbohydrates, 69.42%). When a 0.01% solution of $CoSO_4$ was applied, the quantity of sucrose was 3.94% (all data are given as calculated on the basis of the dry substance) and the quantity of starch 67.2% (sum of the carbohydrates, 71.14%). Still better results were obtained in the case of a $CoSO_4$ solution of lower concentration (0.001%) when the quantity of sucrose decreased to 3.4% and the quantity of starch increased to 68.0% (sum of the carbohydrates, 71.4%).

The data obtained show not only the favorable effect of the microelement Co on the carbohydrate metabolism (this leads to an increase in the quantity of starch and the normal concentration of carbohydrates, which betters the quality of the grain), but also that the lower concentration of the salts of Co^{2+} is more effective. In taking part in certain oxidizing-reducing processes, the microelement helps to increase the activity of a number of enzymes. Our investigations show an increase in the activity in corn leaves of such important oxydases as peroxydase, ascorbinoxydase, and polyphenoloxydase. The oxydases can activate the molecular O_2 and give it the ability to be reduced into H_2O_2 or H_2O in proportion to the hydrogen of the oxidized substance [19]. The concentration of the given salt can play a deciding role because it leads to an increase in the quantity of catalyzed O_2. The presence of small quantities of O_2 in the reactions most often shows an entirely favorable effect, but for those processes, O_2 in large quantities can appear to be an inhibitor.

In the first stages of development of corn 10-15 days after the appearance of the shoots, the activity of the adcorbinoxydase in the leaves with a pH of 7.5 had already increased from 10.6 (control) to 14.2 and after a week these figures were 16.9 and 28.0, respectively. In this manner, the use of a very diluted 0.001% solution of $CoSO_4$ noticeably increased the activity of the ascorbinoxydase.

A few other results were obtained when the activity of polyphenoloxydase was determined. With this, the significance of the pH, the activity of the indicated enzyme in the leaves in the first stages of development was 66.1 for the control and 58.0 for the Co^{2+}. However, after a week, these figures were already 37.7 and 57.2, respectively. With a pH of 5.0, the activity of the enzymes under the action of the microelement was always higher.

The activity of the peroxydase in the corn leaves in the first stages of development increased from 87.9 to 136.7 (Co^{2+}) and after a week to 144.3 and 214.5, respectively.* For all of the observed oxidizing-reducing

*The activity of the enzymes everywhere is expressed in g of oxidized ascorbic acid per 1 g of tissue.

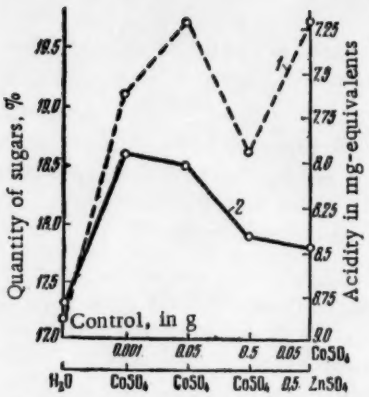

Fig. 1. The effect of different concentrations of the microelement Co and its combinations with the microelement Zn (in g per bush) on the quantity of sugar and the acidity of grape, variety Aligote, with root feeding. 1) quantity of sugar in %; 2) titrated acidity in g/l.

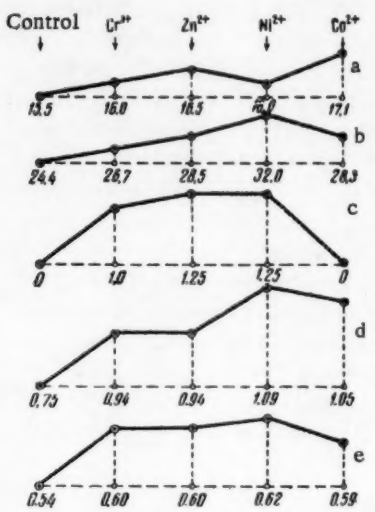

Fig. 2. The effect of microfertilizers on several processes taking place in grape. a) Oxidizability of the tissue (ml $\frac{H}{300}$ I_2); b) oxidizability of the tissue (ml $\frac{H}{300}$ KMn O_4); c) activity of the polyphenoloxydase in the grapes; d) glucoacidimetric indicator: e) proportion of fructose to glucose

enzymes, there was characteristically an increase in the activity in the beginning and then a decrease in proportion to the development of the plants. Together with this, this activity is retained for a longer period under the effect of microelements.

In these same experiments, the use for preplanting trestment of seeds of a solution containing simultaneously Co^{2+} and Mn^{2+} (0.001% solution of $CoSO_4$ and a 0.01% $MnSO_4$, giving favorable results separately) did not lead to better carbohydrate metabolism for corn. The quantity of sucrose was noticeably decreased to 2.82%, and the quantity of starch was 64.8%. Thus, the sum of the carbohydrates was 67.62%; that is, it was even less than in the case of the control. A similar unfavorable effect of combinations of several microelements observed also in other cases was called by us resistance of the microelements.

An increase in the yield and in grape sugar and a decrease in their acidity was observed with the use for a number of years in grape culture of microfertilizers [6, 13] containing in their composition salts of Co^{2+}, Ni^{2+}, Cd^{2+} and Cr^{3+}. For example, as we can see from Fig. 1, different dosages of $CoSO_4$ favorably affected the accumulation of sugar in grapes and the reduction in the titrated acidity of the must. The indicated microfertilizers change the importance of the Eh in the leaves and grapes and, as is clear from Fig. 2, increase in this manner the oxidizability of the tissues which leads to a growth in the activity of a number of enzymes (in particular, polyphenoloxidase), and to an acceleration of the ripening of the grapes because the glucoacidimetric indicator is increased, and the increase of sugars proceeds in large part in proportion to the fructose.

The foliar application of solutions containing sulfates of Cd^{2+}, Hg^{2+}, Ti^{3+}, Cr^{3+}, Co^{2+}, and Ni^{2+} to various agricultural crops also appeared to be very effective. This was shown for many vegetable and grain crops using some of the microelements and for grape using all of the listed microelements. For example, the salts of Co^{2+} increased the weight of grapes (Fig. 3 and 4) and decreased the titrated acidity of the grapes (Fig. 5). The favorable effect of microelements applied at the rate of 10^{-2} to 10^{-5} g of sulfate per grape bush is clearly seen in the example of the growth of saccharinity of the grapes: 3.3% (Cd^{2+}), 3.05% (Hg^{2+}), 1.8% (Ti^{3+}), 2.45% (V^{2+}), 2.5% (Cr^{3+}), 1.85% (Co^{2+}) and 2.5% (Ni^{2+}). In all cases, an increase in the weight of the grapes and the yield was noted.

Such effectiveness of the observed microelements is explained by changes in a number of oxidizing-reducing processes in the leaves and fruits of grape. Thus, under the effect of salts of Ti^{3+} a decrease takes place in

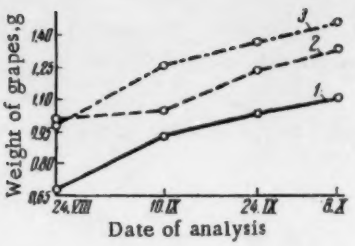

Fig. 3. Dynamics of the change in weight of grapes on foliar application of the microelement Co: 1) control; 2) $CoSO_4$; 3) $CoSO_4$ + boric fluid.

the value of the Eh of the leaves from 0.531 to 0.489 volts with a decrease in the value of the pH from 4.95 to 4.58. All of this led to an increased concentration of ascorbic acid in the leaves in the various stages of vegetation [for Cr^{3+}, Fig. 4 (A)] which was accompanied by a growth in the activity of the most important oxidizing-reducing enzymes. This can be traced, for example, for such a microelement which increases, beginning from the moment of use up to the end of vegetation, the activity of the ascorbinoxydase [Fig. 4 (B)] the activity of polyphenoloxydase [Fig. 4 (C)] and the activity of peroxydase [Fig. 4 (D)]. The noticeable effect of the microelements Zn and Co on the activity of the peroxydase is seen in Fig. 5.

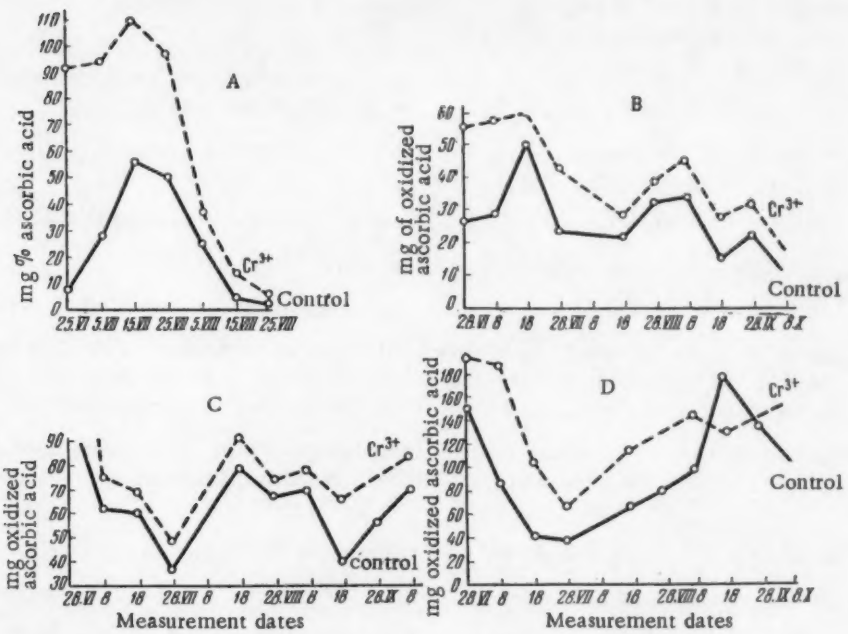

Fig. 4. The effect of the microelement Cr on the concentration of ascorbic acid (A) the activity of the ascorbinoxydase (B), the activity of the polyphenoloxydase (C), and the activity of the peroxydase (D) in the leaves of grape, variety Riesling, with a pH of 7.5.

The number of examples showing the various sides of the action of the observed, but little studied, microelements on the oxidizing-reducing processes, enzyme activity, and carbohydrate metabolism could be increased many times.

It must be assumed that with the help of microelements, one can positively affect the various processes taking place in plants. The number of poorly studied or even unstudied microelements is very high and they can serve as important factors of controlled changes in the development of organisms.

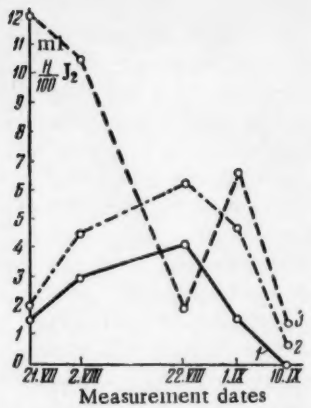

Fig. 5. The effect of the microelements zinc and cobalt on the activity of peroxydase in grape leaves upon foliar feeding; 1) control; 2) $CoSO_4$; 3) $ZnSO_4$.

LITERATURE CITED

[1] O. K. Dobrolyubskii, Microelements in Agriculture [in Russian] (Sel'khozgiz, 1956).

[2] S. A. Voznesenskii, Transactions of the All-Union Conference on Analytical Chemistry [in Russian] (Izd. AN SSSR, 1939).

[3] A. O. Voinar, The Biological Role of Microelements in the Organism of Animals and Man [in Russian] (Izd. "Sov. Nauka", 1953).

[4] K. Scharrer, Biochemie der Spurenelemente. (Verl. P. Parey, Berlin and Hamburg, 1955).

[5] E. L. Spenger, Amer. J. Bot. 24, 16 (1937).

[6] O. K. Dobrolyubskii and A. V. Slavvo, Doklady Akad. Nauk SSSR 117, 6, 1064 (1957)*; Doklady Akad. Nauk SSSR 118, 5, 1040 (1958).*

[7] O. K. Dobrolyubskii and A. V. Slavvo, Doklady Akad. Nauk SSSR 106, 4, 735 (1956); Tr. Odesskogo S.-kh. Inst. 8 (1957); 13 (1958).

[8] O. K. Dobrolyubskii, VIII Mendeleev Congress on Ordinary and Applied Chemistry (Collection of authors' papers and notes) No. 7 [in Russian] (Izd. AN SSSR, 1958).

[9] G. Neilands and P. Stumpf, Essays on the Chemistry of Enzymes [Russian translation] (IL, 1958).

[10] M. Ya. Shkol'nik and S. A. Abdyrashitov, Fiziol. Rastenii 5, 393 (1958).*

[11] M. Ya. Shkol'nik and N. A. Makarova, Microelements in Agriculture [in Russian] (Izd. AN SSSR, 1957).

[12] O. K. Dobrolyubskii, Collection of Thesis Reports, III All-Union Conference on Microelements [in Russian] (Izd. AN AzerbSSR, Baku, 1958); Doklady Akad. Nauk SSSR, 101, 6, 1135 (1955); Collection, Microelements in Agriculture and Medicine [in Russian] (Izd. AN LatvSSR, 1956); Vinodelie i Vinogradarstvo SSSR, No. 3, 19 (1957).

[13] O. K. Dobrolyubskii and A. V. Slavvo, Doklady Akad. Nauk SSSR, 100, No. 3 (1955); Doklady Akad. Nauk SSSR, 112, No. 2 (1957)*; Udobrenie i Urozhai, No. 2 (1958); Sadovodstvo, Vinogradarstvo i Vinodelie Moldavii, No. 5 (1958).

[14] E. J. Haertl and A. E. Martell, J. Agric and Food Chem. 4, No. 1 (1956).

[15] A. Najjar, The Role of Metal Ions in Enzyme Systems. "Phosphorus metabolism". (Baltimore, 1951).

[16] R. H. Kenten and P. J. G. Mann, Biochem. J. 50, 360 (1950).

[17] H. Herzmann, Urania, 20, No. 2 (1957).

[18] D. Schemin and C. S. Russell, J. Amer. Chem. Soc. 75, 487 (1940).

[19] D. M. Mikhlin, Biological Oxidation [in Russian] (Izd. AN SSSR, 1956).

[20] M. Ya. Shkol'nik, Collection. Microelements in Agriculture and Medicine [in Russian] (Izd. AN LatvSSR, 1956).

[21] O. K. Dobrolyubskii, Research in the Area of Complex Cyanides of Cobalt [in Russian] (Institute of Ordinary and Nonorganic Chemistry, Akad. Nauk UkrSSR, Kiev, 1952).

[22] O. K. Dobrolyubskii and A. V. Slavvo, Tr. Odesskogo S.-kh. 13, 111 (1958).

[23] F. Seitz, The Modern Theory of Solids. (New York, London, 1940).

* See English translation.

[24] W. H. Pearsall, J. Ecol. 26, 194 (1938).

[25] J. Small, Modern Aspects of pH (London, 1954).

[26] O. K. Dobrolyubskii, Priroda, 10, 85 (1956).

[27] O. K. Dobrolyubskii, Tr. Odesskogs S.-kh. Inst. 13, 103 (1958).

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THE LOCALIZATION OF MANGANESE IN VARIOUS CELL STRUCTURES OF PLANTS

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The physiological significance of the microelement manganese is connected with the formation of metallo-organic compounds, which play the role of strong catalyzers in plants.

Compounds of manganese with enzymes, which result in the strengthening of the oxidizing-reducing processes in plants, have great significance for intercellular metabolism.

Manganese is a component part of the enzyme arginase. Besides this, manganese is an active catalyst for dicarboxylase of oxalacetic acid. It activated phosphoglucomutase, enolase, arginase, lecetinase, aminopeptidase, and other enzymatic systems.

In our experiments, the connections of manganese with protein complexes in the seeds of a number of agricultural plants that differ in the character of their metabolism were studied, and the stability of the connection of manganese in plant structures based on the quantity and order of its digestion by different solutions was also established.

The connection of manganese with protein complexes is shown to us by the presence of certain manganese-organic and adsorption complexes. Among sources in the literature on this question is the work of Mayer [1], who determined the concentration of iron, manganese, and copper in lettuce seeds in water soluble compounds and proteins precipitated from the water solution with alcohol, and who showed that the concentration of iron, manganese, and copper in compounds with the proteins are significantly less than their concentration in the water soluble fraction. Shakhova [2] under conditions of a boracic biogeochemical province showed a correlation of boron to the carbohydrate fraction; the fatty fraction contains boron in an insignificant quantity and the protein fraction is free of boron.

In our research on the connection between manganese and the protein complexes, we used the seeds of plants in which proteins predominate (peas, beans, lupine), the seeds of plants in which starch predominates (corn, winter wheat, buckwheat), and the seeds of plants in which oil predominates (sunflower, flax).

There was a triple extraction of proteins from a 20 g batch of ground seeds in the rotator for a period of an hour as follows: with water, with a 5% solution of potassium sulfate, with a 0.2% solution of sodium hydroxide, and with alcohol. After being filtered in filtrates, each fraction of the protein was precipitated with 10% trichloroacetic acid and tannin. The precipitates of the proteins were filtered off and washed with 1% trichloroacetic acid.

The dried precipitates of the proteins were burned and the concentration of manganese in them was determined according to the method of Vlasyuk and Gorna [3]. After this, the extracted filtrates were evaporated until dry, the dry remains were burned and the manganese in the filtrates and the dry remains were also determined.

The data on the connection between manganese and the proteins, and between the stability of its connection and the cell structures of winter wheat, corn and buckwheat, are given in Table 1.

TABLE 1

The Concentration of Manganese in the Proteins and Filtrates Extracted from the Seeds of Winter Wheat, Corn and Buckwheat (as % of dry substance).

Crop	Study objects	Manganese in fractions				
		water	salt	alkaline	alcohol	dry remains
Winter wheat	protein	—	—	—	$2.1 \cdot 10^{-4}$	—
	filtrate	$1.32 \cdot 10^{-3}$	$1.23 \cdot 10^{-3}$	$5.8 \cdot 10^{-4}$	$3.5 \cdot 10^{-4}$	$8.5 \cdot 10^{-4}$
Corn	protein	—	—	—	$2.8 \cdot 10^{-4}$	—
	filtrate	$1.25 \cdot 10^{-3}$	$2.5 \cdot 10^{-3}$	$3.5 \cdot 10^{-4}$	$7.6 \cdot 10^{-4}$	$1.13 \cdot 10^{-3}$
Buckwheat	protein	—	—	—	—	—
	filtrate	$4.88 \cdot 10^{-3}$	$5.80 \cdot 10^{-4}$	$5.80 \cdot 10^{-4}$	$2.10 \cdot 10^{-4}$	$2.93 \cdot 10^{-4}$

The indicated data bear witness to the fact that manganese was found in the protein fraction that was precipitated with alcohol in the seeds of winter wheat. Manganese was not found in the remaining protein fractions. The remaining fractions are distributed in this decreasing order: water > salt > alkaline > alcohol, based on the concentration of manganese extracted by various solutions. Some quantity of the manganese is found in the dry remains of the seeds after extraction by various solutions, which attests to its stable connection with the cellular structures of winter wheat (membranes, cellulose).

In corn seeds, as in the seeds of winter wheat, manganese is to some degree connected with the proteins precipitated by alcohol and it was not found in the remaining protein fractions. Based on the quantity of manganese extracted by the different solutions, the remaining fractions from the corn seeds are found in the following descending order: salt > water > alcohol > alkaline. The high concentration of manganese in the dry remains from the corn seeds attests to the fact that it is connected here in a stable manner with the cell menbranes.

We found support for our data in the investigations of Schoenleber 4] who, in experiments with Holodea and Caulerpa over a period of three years, showed that manganese is deposited in the form of conglomerations or in the form of separate layers in the membranes of the assimilating cells; in both cases it is localized in the middle part of the exterior walls of the cells.

Buckwheat seeds, as is well known, direct the accumulation of manganese by the plants.

However, as we can see from the data in Table 1, manganese was not found in a single of the protein fractions of buckwheat seeds; its greatest quantity was extracted by the water extract and the salt and alkaline extracts were practically the same. In the alcohol extract and in the dry remains of the buckwheat seeds, an insignificant, almost a trace, quantity of manganese was found. Thus, manganese is found in the buckwheat seeds in a more mobile condition and is weakly connected with the cell structures. The concentration of

TABLE 2

The Concentration of Manganese in the Proteins of the Seeds of Pea, Bean, Lupine, Flax, and Sunflower in Various Fractions (as % of dry substance)

Crops	Water		Salt		Alkaline		Alcohol		Dry remains
	protein	filtrate	protein	filtrate	protein	filtrate	protein	filtrate	
Pea	—	$1.73 \cdot 10^{-3}$	—	$5.4 \cdot 10^{-4}$	—	$7.1 \cdot 10^{-4}$	—	$6.3 \cdot 10^{-4}$	—
Bean	—	$2.2 \cdot 10^{-3}$	—	$1.01 \cdot 10^{-3}$	—	$3.5 \cdot 10^{-4}$	—	—	—
Lupine	—	$2.07 \cdot 10^{-2}$	—	$8.13 \cdot 10^{-4}$	—	$8.75 \cdot 10^{-4}$	—	$1.18 \cdot 10^{-3}$	$7.6 \cdot 10^{-4}$
Flax	—	$2 \cdot 10^{-3}$	—	$1.36 \cdot 10^{-3}$	—	$1.10 \cdot 10^{-3}$	—	$1.02 \cdot 10^{-4}$	$7.6 \cdot 10^{-4}$
Sunflower	—	$2.18 \cdot 10^{-3}$	—	$8.2 \cdot 10^{-4}$	—	$2.76 \cdot 10^{-3}$	—	$3.81 \cdot 10^{-4}$	$3.1 \cdot 10^{-4}$

manganese in the seeds of protein plants (pea, bean, lupine) and in oil plants (flax, sunflower) and its stability in connection with the cell structures are given in Table 2.

It follows from the data in Table 2 that manganese is not connected to any degree with the proteins in the seeds of protein plants (pea, bean, lupine). The greatest quantity of it for all of the legume crops was found in the filtrate of the water fraction. Second place, based on the concentration of manganese, is taken by the salt an and alkaline fractions. Manganese was not observed at all in the filtrate of the alcohol fraction of the bean seeds. Also, manganese was not found in the dry remains of pea and bean after the extraction of the protein, but it was found in an insignificant quantity in the dry remains of lupine. Thus, in the seeds of legume plants, the microelement manganese is weakly connected with the elements of the cell structure.

TABLE 3

The Concentration of Manganese in the Coagulate and in the Fluids Remaining after Coagulation of the Extract from the Leaves of Sugar Beet (as % of the dry substance)

Experimental scheme—nutrient mixture in all variants and the supplement	Coagulate	Fluid remaining after coagulation
Boron in the feeding	Trace	$1.0 \cdot 10^{-3}$
Boron at the beginning of vegetation	Trace	$1.1 \cdot 10^{-3}$
Manganese at the beginning of vegetation + boron in the feeding	Trace	$1.8 \cdot 10^{-3}$
Manganese + boron at the beginning of vegetation	$1.2 \cdot 10^{-3}$	$1.8 \cdot 10^{-3}$
Manganese + molybdenum at the beginning of vegetation + boron in the feeding	1.10^{-3}	$1.7 \cdot 10^{-3}$
Zinc at the beginning of vegetation + boron in the feeding	$7.6 \cdot 10^{-4}$	$1.3 \cdot 10^{-3}$

Manganese was not found in the proteins from the seeds of oil plants; a small quantity of it appeared in the water fraction of the flax seeds. There were almost equal quantities of manganese in the salt, alkaline, and alcohol fractions; a significant quantity of it was found in the dry remains and this suggests a very stable connection between the manganese and the cell membranes. The separate fractions were distributed in the following decreasing order: alkaline > water > salt > alcohol, based on the quantity of manganese in sunflower seeds extracted with different solutions.

When the proteins and filtrates from the leaves of winter wheat and sugar beets grown under nutrient conditions with high doses of manganese (nutrient mixture + three doses of sulfate of manganese) were studied, the concentration of the latter (as % of the dry substance) in the fractions from the leaves of the indicated crops was as follows: manganese was absent in the protein of winter wheat and $1.45 \cdot 10^{-2}$ in the filtrate; $2.80 \cdot 10^{-4}$ in the protein of sugar beet, and $1.54 \cdot 10^{-1}$ in the filtrate.

The indicated data show that a noticeable quantity of manganese is concentrated in the isolated proteins from the sugar beet leaves. The determination of the concentration of manganese in coagulates obtained by coagulation of the cell sap of sugar beet leaves at a temperature of 81° and in the fluids remaining after coagulation indicated the fact that a smaller quantity of manganese is found in the coagulate than in the fluid, as the data in Table 3 shows.

The data given in Table 3 on the character of the connection between manganese and the proteins bear witness to its concentration in plants in the ion form, which can go into solution under the effect of various solvents; however, manganese was also found in the composition of several protein fractions. Mainly, it is found in the composition of the protein fraction from the seeds of winter wheat and corn, but it is also found in the composition of the total protein fraction extracted from the leaves of sugar beet. Less manganese is found in the coagulate from the sugar beet leaves than in the fluid remaining after coagulation.

TABLE 4

The Concentration of Manganese in the Chloroplasts and Filtrate of the Leaves of Bean in Relation to the Conditions of Manganese Nutrition (as % of the moist substance)

Experimental scheme	Weight of 100 plants, g	Normal concentration of manganese	Concentration of manganese as % of moist substances		Concentration of manganese in the chloroplasts as % of normal quantity
			in the filtrate	in the chloroplasts	
Knop nutrient medium without manganese	269	$1.36 \cdot 10^{-3}$	$9.36 \cdot 10^{-4}$	$4.3 \cdot 10^{-4}$	31.6
Same + manganese	400	$2.53 \cdot 10^{-3}$	$2.02 \cdot 10^{-3}$	$5 \cdot 10^{-4}$	19.8

Among the several protein fractions, manganese results in the formation of metalloorganic complexes which play an important role in photosynthesis and other synthesizing processes. We also studied the localization of manganese in the chloroplasts of several agricultural plants.

The great significance of the microelement manganese in the regulation of the oxidizing-reducing processes in the plant organisms in relation to the conditions of their nutrition was established by the work of one of the authors (Vlasyuk [3]). Manganese is a strong reducer with nitrate feeding, but it is a strong oxidizer with ammonium feeding.

We can assume from the fact that the process of photosynthesis is also an oxidizing-reducing process that manganese shows a positive effect on the intensity of the formation and the rate of movement of the assimilates formed in the process of photosynthesis.

The effect of a number of microelements, including the microelement manganese, on the intensity of photosynthesis under conditions of foliar feeding of plants was confirmed by the work of Ostrovskaya [5]. In a period of 4 days after foliar feeding with a 0.05% solution of sulfate of manganese, the absorption of carbon dioxide by the leaves of sugar beet increased 12, 29, 32 and 12%.

Simultaneously, with Kosmatyi [6], we established with the help of the tracer method the fact that the flow of the products of photosynthesis from the leaves of sugar beet into the roots was noticeably strengthened under the effect of manganese. The significance of manganese for the process of photosynthesis is apparently connected with the fact that it activates the reversible carboxylization of di- and tricarbonic acids.

As a result of the fact that the chloroplasts are significant in the processes of the oxidization of manganese, we studied its concentration in the chloroplasts of plants with different types of metabolism (bean, corn, sugar beet) in relation to the conditions of nitrogen and manganese nutrition.

The concentration of copper, iron, zinc and manganese in the chloroplasts of spinach is mentioned in Vecher's works. This agrees with the data here, which show an average of 80% of the iron to be held in the tissues of the leaves, up to 65-70% of the zinc, about 50% of the copper, and also 45.8% of the manganese, and also agrees with the data of Sisakyan and Kobyakova [7], and establishes the presence of different enzymes, which contain in the main iron and copper, in the chloroplasts.

In our investigations the chloroplasts were separated according to the method of Shlyk and Godnev [8]. The manganese in the chloroplasts and nongreen cytoplasmatic structures was determined according to the method of Vlasyuk and Gorna. A short experiment on the study of the concentration of manganese in the chloroplasts in relation to its presence in the nutrient medium was carried out with the leaves of plants with different types of metabolism: with protein plants (bean) and starch plants (corn).

The plants were grown in a quartz sand, which did not contain manganese, on a Knop nutrient medium with the following scheme: Knop medium (control) and Knop medium + manganese.

TABLE 5

The Concentration of Manganese (in %) in the Chloroplasts and Filtrate of Corn Leaves in Relation to the Conditions of Manganese Nutrition

Experimental scheme	Weight of 100 plants, g	Normal concentration of manganese in moist substance	Concentration of manganese		Concentration of manganese in the chloroplasts as % of normal quantity
			in the filtrate	in the chloroplasts	
Knop nutrient medium without manganese	250	$8.32 \cdot 10^{-4}$	$6.6 \cdot 10^{-4}$	$1.72 \cdot 10^{-4}$	20.4
Same + manganese	277	$2.06 \cdot 10^{-3}$	$1.7 \cdot 10^{-3}$	$3.6 \cdot 10^{-4}$	17.4

Planting was done on April 3 and the experimental plants were harvested on May 25. Data for the study of the effect of manganese on the growth and development of bean plants, and also the concentration of manganese in the chloroplasts and in the filtrate are given in Table 4.

It follows from the data in Table 4 that bean reacts positively to the presence of manganese in the nutrient medium and significantly increases its vegetative mass. Manganese from the nutrient medium, moving intensively into the bean plants, aids in the increase of its concentration both in the nongreen cytoplasmatic structures (filtrate) and in the chloroplasts; here it appeared greater in the filtrate than in the chloroplasts.

We must stress that the concentration of manganese in the chloroplasts as a percent of its normal quantity was found to be higher in the case where the nutrient medium did not contain manganese. This shows that with respect to the selectivity of the chloroplasts in relation to manganese, there is apparently a certain level above which manganese can be toxic. The data on the effect of manganese on the growth and development of corn, and also on its concentration in the chloroplasts and filtrate of corn, are given in Table 5.

The results of the experiment with corn show the same rules for the distribution of manganese between the chloroplasts and the filtrate as were noted for bean.

Manganese from the nutrient medium moved intensively into corn plants; the normal concentration of manganese both in nongreen cytoplasmatic structures and in the chloroplasts increased; however, its highest concentration (as % of its normal quantity) was found in the chloroplasts of the control plants.

Experiments on the combined action of the microelements in conditions of a normal and an inadequate supply to plants of nitrogen were carried out in sand cultures with sugar beet against a background of boron. The concentration of manganese in the chloroplasts and filtrate was determined in relation to the conditions of manganese nutrition (Table 6).

It follows from the data in Table 6 that the principal quantity of manganese entering into the plants is concentrated in the nongreen cytoplasmatic structures. With a low level of nitrogen nutrition, the intake of manganese was decreased. In the middle of vegetation, the quantity of manganese entering into the filtrate and chloroplasts, and also the normal concentration of manganese in the sugar beet, increased in comparison with the beginning of vegetation. When manganese was introduced, its normal quantity in the filtrate and in the chloroplasts increased; however, it was found more in the chloroplasts of control plants.

SUMMARY

1. The presence of manganese in the proteins of the alcohol fraction from the seeds of winter wheat and corn, and also in the total of the precipitated proteins from the leaves of sugar beet, was established. Manganese was not found in the proteins of the seeds of buckwheat, pea, bean, lupine, flax and sunflower.

2. The predominant quantity of manganese in sugar beet leaves was localized in the cell sap; there was less in the coagulates.

TABLE 6

Concentration of Manganese in the Chloroplasts and Filtrate of Sugar Beet, in Relation to the Conditions of Nitrogen and Manganese Nutrition (as % of moist substance)

Experimental scheme	May 27				June 1			
	concentration of manganese	concentration of manganese		concentration of manganese in the chloroplasts as % of normal concentration	concentration of manganese	concentration of manganese		concentration of manganese in the chloroplasts as % of normal concentration
		in the filtrate	in the chloroplast			in the filtrates	in the chloroplasts	
Nutrient medium without microelements	$5.9 \cdot 10^{-4}$	$3 \cdot 10^{-4}$	$2.9 \cdot 10^{-4}$	49.1	$1.22 \cdot 10^{-3}$	$5.93 \cdot 10^{-4}$	$6.35 \cdot 10^{-4}$	51.7
Same + manganese	$1.04 \cdot 10^{-3}$	$8.16 \cdot 10^{-4}$	$2.30 \cdot 10^{-4}$	22.1	$2.07 \cdot 10^{-3}$	$1.72 \cdot 10^{-3}$	$3.50 \cdot 10^{-4}$	16.9
Same + manganese + copper	$9.66 \cdot 10^{-4}$	$7.26 \cdot 10^{-4}$	$2.40 \cdot 10^{-4}$	25.0	$1.61 \cdot 10^{-3}$	$1.23 \cdot 10^{-3}$	$3.81 \cdot 10^{-4}$	23.7
Same + manganese + zinc	$1.02 \cdot 10^{-3}$	$7.80 \cdot 10^{-4}$	$2.40 \cdot 10^{-4}$	23.5	$1.76 \cdot 10^{-3}$	$1.26 \cdot 10^{-3}$	$5.06 \cdot 10^{-4}$	28.4
Same with a half dose of nitrogen without manganese	$6.49 \cdot 10^{-4}$	$3.53 \cdot 10^{-4}$	$2.96 \cdot 10^{-4}$	45.6	$9.56 \cdot 10^{-4}$	$6.66 \cdot 10^{-4}$	$2.90 \cdot 10^{-4}$	30.5
With half dose of nitrogen + manganese + copper	$8.05 \cdot 10^{-4}$	$6.10 \cdot 10^{-4}$	$1.95 \cdot 10^{-4}$	24.2	$1.86 \cdot 10^{-3}$	$1.38 \cdot 10^{-3}$	$4.81 \cdot 10^{-4}$	25.8
With half dose of nitrogen + manganese + zinc	$9.66 \cdot 10^{-4}$	$7.26 \cdot 10^{-4}$	$2.40 \cdot 10^{-4}$	25.0	$1.66 \cdot 10^{-3}$	$1.31 \cdot 10^{-3}$	$3.5 \cdot 10^{-4}$	21.0
	$8.73 \cdot 10^{-4}$	$6.83 \cdot 10^{-4}$	$2.40 \cdot 10^{-4}$	27.5	—	—	—	—

3. The greatest stability in the connection of manganese and the cell structure, which was determined by its concentration in the dry remains after precipitation of the proteins, showed up for seeds of winter wheat and was significantly less in the seeds of buckwheat, pea, bean, and lupine.

4. The main quantity of manganese in the seeds of starchy, legume, and oil plants, which was easily extracted by different solvents and is instrumental in the activation of a number of metalloenzymes, was found in the ion form.

5. Most of the manganese is localized in the nongreen cytoplasmatic structures; a smaller quantity of manganese was found in the chloroplasts.

6. When manganese was applied in the nutrient medium, its concentration in the filtrate and chloroplasts increased; however, it was greatest in the chloroplasts of control plants. The idea of the presence of a certain level of selectivity of the chloroplasts in relation to manganese is expressed.

7. With a lower level of nitrogen nutrition at the beginning of vegetation of sugar beets, the normal concentration of manganese in the plants and in the filtrate decreased, while its presence in the chloroplasts did not change with various levels of nitrogen nutrition.

LITERATURE CITED

[1] A. M. Mayer, Physiol. plantarum, 7, 4, Collection of Reports, Moscow (1957).

[2] I. K. Shakhova, Thesis Report on the Conference on the Biogeochemical Province [in Russian] (Izd. AN USSR, Kiev, 1957).

[3] P. A. Vlasyuk, New Properties of Manganese [in Ukrainian] (AN UkSSR, Kiev, 1940).

[4] K. Schoenleber, Protoplasma 27, 4 (1957).

[5] L. K. Ostrovskaya, Second Conference on Photosynthesis Thesis of Reports [in Russian] (Izd. MGU, 1957).

[6] P. A. Vlasyuk, Z. M. Klimovitskaya and E. S. Kosmatyi, Trudy Ukr. N.-I. Inst. Fiziol. Rastenii, No. 13-14 (1958).

[7] I. M. Sisakyan and A. M. Kobyakova, Biokhimiya 86, 14 (1949).

[8] A. A. Shlyk and M. N. Godnev, Bibliography of the Scientific Research Works of the Institute of Biology, Akad. Nauk BSSR [in Russian] (1956).

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THE BEHAVIOR OF GREEN PLASTIDS IN THE CORTEX OF LONG AND SHORT SHOOTS OF SEVERAL FRUIT TREES UNDER CONDITIONS OF A DRY SUBTROPICAL CLIMATE

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The occurrence and formation of green plastids in the "nonassimilating organs" have resulted in a great deal of interest being shown by morphologists and physiologists.

The presence and formation of green plastids in the cortex, wood, and shrub fruits, in the young shoots, and even in the cells of the pith, has been established by the investigations of a number of authors [1-20].

Deneke [21], and Aleksandrov and Savchenko [1], think that the chloroplasts of nonassimilating organs can take an active part in the polymerization into starch of soluble carbohydrates flowing from neighboring cell cells. Protsenko and Polishchuk [21] consider that the chloroplasts of the phelloderm of fruit trees play a role in the frost resistance of the plant, in which the condition of the plastids in the indicated tissue, according to the authors, is an indicator of the degree of frost resistance of the tree.

According to N. V. Tsitsin, the green plastids play an important role in the growing together of the graft and the wilding. A number of other investigators [9, 12, 14-16] view chloroplasts as very mobile systems that can reorganize their apparatus to fulfill one or another function.

The anatomical investigations that we carried out on the condition of the green plastids in the phelloderm cells, wood, and medullary rays of fruit and vegetative shoots of apple, pear and apricot demonstrate very interesting features of the nature of green plastids which is liable to extreme variability over the period of a year.

METHODS

Both northern varieties and representatives of southern fruit plants were used to make an investigation of the condition of green plastids in autumn, winter and spring periods in Kirovabad. In particular, the following served as objects for investigation: apple, variety Astrakhanskoe Krasnoe; pear, varieties Shalakh and Gyshar-mudu; apricot, variety Bul'bon. The objects for investigation were taken from healthy trees of the same age grown under good agricultural care.

Parts of 1-to 5-year-old short and long shoots were taken for investigation. The material was placed in ordinary tap water without using indicators. By doing this we hoped to carry out the investigation of plastids with the elimination of the undesirable deforming effects of indicators. Cuts prepared by hand were placed in a 10% solution of sucrose and examined under the microscope.

Investigations were carried out systematically every 2-3 weeks. Sections for investigation were prepared in a similar manner for both vegetative and fruiting shoots of the above-named plants.

For the determination of the presence of starch in the cells, sections were treated with iodine and potassium iodide. The most characteristic pictures, demonstrating the condition of the variability of the plastids, were obtained with the help of the Abbé apparatus.

RESULTS OF THE INVESTIGATION

The entire process of change in the condition of the green plastids in the phelloderm cells of apple, pear and apricot, as we established in the process of investigation, presents the following picture.

Autumn period. There are clear green, normal plastids rich in starch in the pehlloderm cells in all cases in September and October. (Fig. 1, a). In November, when the leaves had not yet been shed on apple and pear and the leaves had a green color, the chloroplasts in the cells of the phelloderm still retain normal structure. The beginning of the conglomeration of plastids in threes and fours was already observable in several phelloderm cells that were neighboring on the basic parenchyma cortex (Fig. 1, b). The cells in this period are filled with starch grains and the green plastids are almost completely covered with them. The described condition of the plastids at the time of the investigation was characteristic for the one to three-year-old shoots both for apple and for pear. The shedding of the leaves for the typically southern apricot in this period had already started and the plastids had changed into a condition of agglutination (Fig. 1, c). The process of agglutination in the cell of the phelloderm of apricot is accompanied by hydrolysis of the starch. The agglutinated mass of the chloroplasts gradually intermix with the protoplasm, as a result of which the chlorophyll gradually becomes equally distributed throughout the entire mass of the plasma. However, a portion of the chloroplasts still have a grainy structure and there is starch in them.

The hydrolysis of starch, as noted for apricot in the middle of October, begins for pear toward the end of November on agglutination of the chloroplasts. At this time the process of agglutination of the chloroplasts in the phelloderm cells of apricot reaches a maximum. Starch grains in an insignificant quantity were preserved in each cell, but their size was small. The agglutinated mass in the cells is homogenous and the chlorophyll is distributed equally; the location of the agglutinated mass in some cases is in the center of the cells and in others on each side of the large vacuoles (Fig. 1, d).

Winter period. The process of agglutination encompasses almost all cells in the phelloderm cells of both the one-year-old and three-year-old shoots of pear in December (Fig. 1, e). However, the chlorophyll is distributed unequally over the entire mass of the protoplasm; agglutination in the various cells is in various stages of completion.

Starch grains are also found in the agglutinated mass; however, in the cells of the fruit shoots (short shoots) there are more (Fig. 1, f) because the hydrolysis of the starch starts earlier in growing (long) shoots.

In the one-year-old fruit shoots of apricot the chloroplasts of the phelloderm cells change over completely into the agglutinated condition in the middle of December (Fig. 1, g). Starch grains are absent. In the cells of the two-year-old growth and three-year-old fruit shoots of apricot, an insignificant quantity of starch grains is found in an agglutinized mass, for which the starch grains of the three-year-old shoot are of somewhat greater dimensions. In several cells the starch grains in the agglutinated mass are arranged in a circle (Fig. 1,h).

For apple, at the beginning of December, a large number of starch grains are contained in the cells of the fruit shoots independent of the complete agglutination of the chloroplasts (Fig. 1, i), which is not noted in the growth shoots (Fig. 1, j). Toward the end of December the chloroplasts are still more subjected to the process of agglutination: the quantity of starch grains encompassed by the process of hydrolysis is also decreased somewhat. The circular arrangement of the starch grains is also observed for apple. The entire process of agglutination for apple, both in the fruit and growth shoots, from the end of November to the end of December is characterized by a decreased concentration of chlorophyll.

During January and February the chloroplasts continue to be an agglutinated condition. There are also starch grains in the cells of apple and pear, especially in the fruiting shoots. An accumulation of chlorophyll is observed in separate parts of the protoplasm. There are no starch grains in the phelloderm cells of both the growth and fruiting shoots of apricot; chlorophyll is distributed equally through the entire mass of the plasma.

The separation of the parts of the protoplasm rich in chlorophyll can be viewed as the beginning of the process of deagglutination, that is, plastid formation.

Spring period. In March the process of deagglutination begins in full volume. In the phelloderm cells of pear the formation of plastids is started from the agglutinated mass by coagulation of the pigments of the individual parts (Fig. 1, k). The sizes of the similar spots are different. In several of them, starch is accumulated.

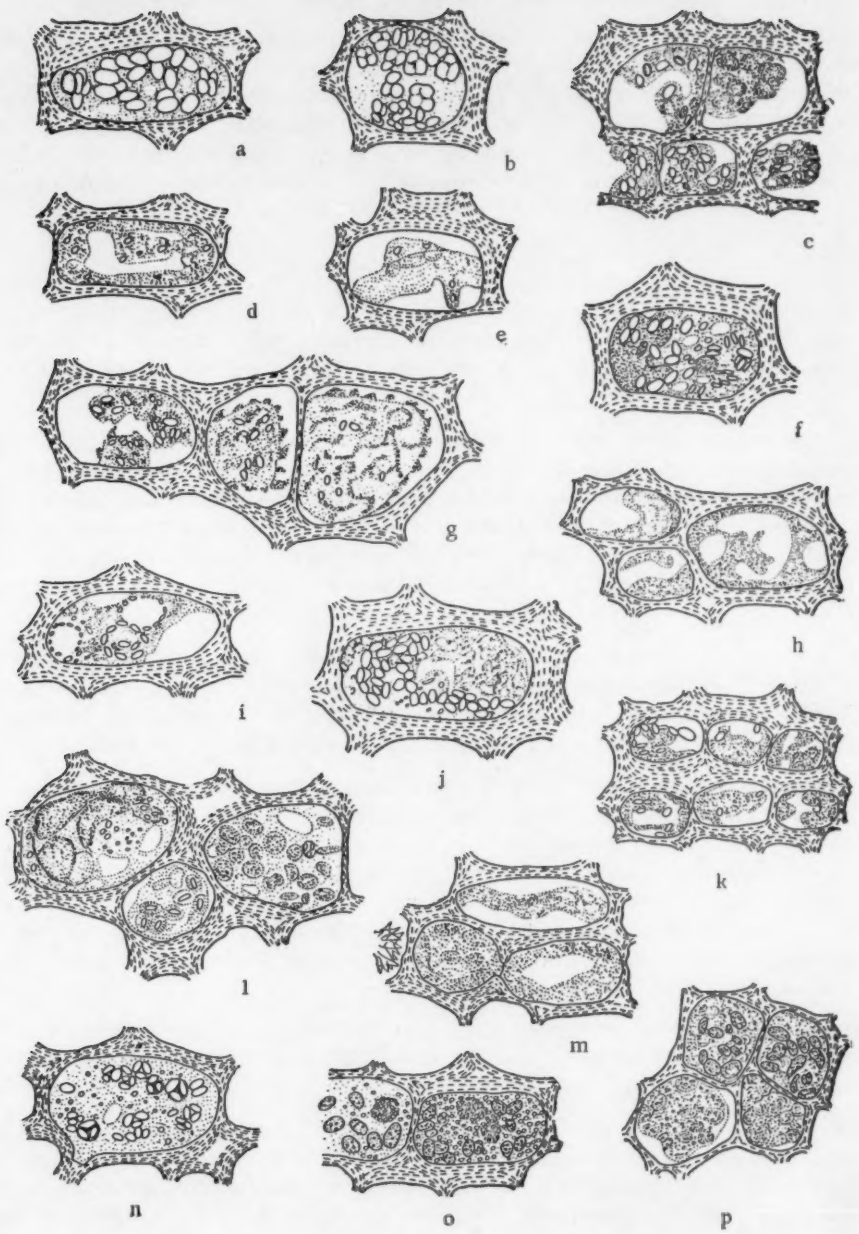

Fig. 1. The change in the condition of the green plastids in the phelloderm cells of apple, pear and apricot (explanation in the text).

Thus, the process of the new formation of the chloroplasts is accompanied by the polymerization of the starch, for which a circular arrangement of the little grains is often observed.

The isolated parts of the agglutinated mass at first have large sizes, then these parts are decreased, the concentration of chlorophyll in them increases, and, finally, plastids of a normal structure are formed. However, the process of deagglutination in the different cells does not proceed simultaneously.

The chloroplasts of apricot continue to be in the agglutinated condition at the beginning of March (Fig. 1, e, f, g, h). The accumulation of chlorophyll in the isolated parts of the agglutinated mass is observed for apricot only from the beginning of March (Fig. 1, l). The chloroplasts are still in a strongly agglutinated condition in the fruiting shoots at the very climax of flowering, a fact also noted in the phellodermal cells found much above (more than 0.5 cm) the base of the peduncle. The starch grains in the indicated cells were subjected to complete hydrolysis. At the time of the blossoming of the buds and the formation of the new leaves, the process of the formation of plastids proceeds with the simultaneous accumulation of starch in the phellodermal cells of the growing shoots.

The different conditions of the phellodermal cells of the growing and fruiting shoots which we have described are based on the different conditions of development of the fruiting and growing shoots. The begining stages of development, which are expressed on the surface by the formation of new leaves and the formation of a new shoot, are noted in the growing shoots. At this time, the fruiting shoots are scattered along with the opened flowers.

The strengthened processes of new formation in the growing shoots is undoubtedly accompanied by a strengthening of energoplastic substances. Parallel to this, the energoplastic substances accumulated after the previous vegetation period are used in the process of flowering in the fruiting shoots and in the flowering shoots. Based on this, the new formation of chloroplasts and the accumulation of energoplastic substances are noted in the cells of the growing shoot at the beginning of its formation. In the cells of the fruiting shoots, on the other hand, a strengthening of the growth of energoplastic substances takes place, but the chloroplasts continue in the agglutinated condition.

It follows from what has been said that the process of new formation of chloroplasts is intimately connected with the processes of development of the entire organism and its individual parts. The new formation of chloroplasts occurs,in the first place, in those parts of the plant that demand a stong movement and mobilization of the energoplastic substances that have a place in the growing parts of the plant.

The investigations of Aleksandrov and Savchenko [1] also established that for many catkin-bearing plants, such as alder, hazelnut and birch, the opening of the catkin takes place on the strong agglutination of the plastid apparatus of the phelloderm cells. According to the indicated investigators, the shift of the plastids from an agglutinated to a normal condition depends in the main part on the development of the growing shoots and leaves. This position can be supported by the data given above on the investigation of the condition of the chloroplasts in the phellodermal cells of the growing shoots at the time of the opening of the leaves, the beginning of growth, and the development of the new shoot, where the strong new formation, the separation of the chloroplasts from the agglutinated mass and the accumulation of starch were noted.

Toward the end of March, the plastid apparatus in the phellodermal cells of the fruiting shoot of apple, in general,is in an agglutinated condition. However, along with this the formation of new plastids from the agglutinated mass is also noted (Fig. 1, m).

The flower shoots at this time still have not opened, but are in a very swollen condition. The agglutinated mass and the successfully separated chloroplasts are pale green. The cells also have starch grains, probably reserves from the previous year. Flowering for apple starts somewhat later than for apricot and the special moments in the condition of the chloroplasts, which were established in a parallel investigation of the phellodermal cells of apricot and apple, are fully apparent in March.

In the growing shoots of apple, the changes in the plastid apparatus connected with the beginning of spring proceed somewhat more intensively than in the fruiting shoots. Almost the entire agglutinated mass in the cells is already separated into individual chloroplasts and the cells are filled again with molded starch grains. In March changes were noticed in the phellodermal cells of the growing and fruiting shoots of pear as of apple.

On the basis of what has been said, we can state that in March, in the south, practically all fruits are characterized by the beginning of the process of deagglutination, that is, the formation of new plastids, in general from the agglutinated mass.

A comparison of the data of the investigation with the phases of development for the fruits that were studied and the conditions of the surrounding environment supported fully the dependence of the phases of development on changes in the living conditions in the different seasons of the year, which shows a direct effect on the direction of the metabolic process.

We explain the differences in the behavior of the plastid apparatus for the three indicated fruits by the difference in their time of flowering. In the conditions of Kirovabad, apricot flowers earliest of all of the three named fruits, then pear (approximately after 20-25 days), and lastly, apple. Consequently, all of the reactions of decomposition and systhesis for apricot take place in a more reduced period of time, and for apple are stretched out; pear occupies an intermediate position. Therefore, for apricot, starch, as one of the forms of reserve of energoplastic substances, is used in the various processes of development earlier than for pear and apple. At the same time, the activation of the metabolic process begins earlier for apricot, beginning with the intensive accumulation of the chlorophyll pigment, after which the normal condition of the chloroplastids is restored toward the end of March.

Toward the beginning of April, the process of deagglutination is completely finished in the phellodermal cells both of the growing and fruiting shoots of pear. In rare cases, cells are observed in which the process of separation of the chloroplasts is somewhat retarded. In the growing shoots, the process of starch accumulation is strengthened. The accumulation of starch takes place directly in the chloroplasts. Two to three starch grains develop at the beginning in each chloroplast (Fig. 1, l).

The lag noted above in the changes in March in the condition of the green plastids for apple and pear, as compared with apricot, is noticeably eliminated in April. In April, the completion of the process of deagglutination and strengthening of starch accumulation are also observed for apple and apricot.

The investigation carried out for all three objects of the condition of the plastid apparatus of the phellodermal cells shows a complete restoration of the chloroplasts after the winter period.

At the beginning of May the chloroplasts of apricot, pear, and apple (in the growing and fruiting shoots) were not only completely formed in the independent organs of the cell, but the accumulation of chlorophyll in them reached the highest levels, whereupon the starch grains in them were practically not observed.

The new formation of chloroplastids from the substance of protoplasm is started in the following manner: First, the plasma begins to turn green and a consolidation of the parts and the formation of grains is observed in the mass of the plasma (Fig 1, n). Subsequently, the grains collected in a cluster combine in a normal mass and chloroplasts are formed. The young grains at first are usually colored a pale green. Only the fully formed chloroplast has a clear green color. Attention must be paid to the great mobility of the grains, especially at the time of their accumulation into a cluster.

The results of the established investigation demonstrate the extreme liability of the plastid apparatus to different changes at different times of the year. It is possible that the transfer of plastids into an agglutinated condition aids in increasing the frost resistance and cold resistance of the plant.

SUMMARY

1. In the autumn with the onset of cold weather, the chloroplasts of the stem of woody fruits shift into an agglutinated condition both in the short and long shoots; this appears as a fusing together of chloroplasts, and subsequently also of the plasma, which is colored green by chlorophyll. Of the fruits subjected to investigation, the process of agglutination for apricot begins in the autumn somewhat earlier than for apple and pear, but the process of deagglutination begins later in spring than for the other two, approximately to the extent that the shedding of the leaves and the flowering is earlier for apricot.

2. The process of deagglutination in the conditions of the southern, dry, subtropical climate begins for almost all fruits at the end of February and reaches its full completion in May.

With the beginning of the process of deagglutination, the new formation of chloroplasts both from the agglutinated mass and anew from the substance of the plasma begins. The theory of Shimper on the succession and individuality of the plastids is disproved by the described position.

3. At the same time that the chloroplasts continue to be in a strongly agglutinated condition in the phellodermal cells of the fruiting shoots at the very climax of flowering, and all starch grains in them were subjected to complete hydrolysis in the growing shoots at the moment of opening of the shoots and the formation of new leaves, the intensified process of new formation of chloroplastids and starch accumulation begins. The indicated difference is shown by the occurrence of fruiting and growing shoots in the different stages of development.

LITERATURE CITED

[1] V. G. Aleksandrov and M. I. Savchenko, Trudy Bot. Inst. im. Komarov AN SSSR, ser. VII, No. 1 (Izd. AN SSSR, 1940).

[2] V. G. Aleksandrov, M. S. Yakovlev and L. V. Klimochkina, Bot. Zhur. 32, No. 4 (1947).

[3] D. M. Bagirov, Uchenie Zap. Kirovabadskogo Ped. Inst. im. G. B Zardabi, No. 3, 29 (1955).

[4] L. P. Breslavets, Izv. Akad. Nauk SSSR, ser. biol., No. 3 (1947).

[5] P. A. Genkel' and E. Z. Oknina, Trudy Inst. Fiziol. Rastenii im. K. A. Timiryazeva AN SSSR, Vol. VI, No. 1 (Izd. AN SSSR, 1948).

[6] T. N. Godnev and M. V. Terent'eva, Doklady Akad. Nauk SSSR 83, 3, 481 (1952).

[7] E. R. Glyubbenet, The Plant and Chlorophyll [in Russian] (Izd. AN SSSR, 1951).

[8] T. F. Zavalishina, Doklady Akad. Nauk SSSR, nov. seriya, 78, 1, 137 (1951).

[9] A. A. Zaitseva, Doklady Akad. Nauk SSSR 27, 8, 854 (1940).

[10] V. O. Kazaryan and E. S. Avundzhyan, Doklady Akad. Nauk SSSR 101, 1, 181 (1955).

[11] V. N. Lyubimenko, Photosynthesis and Chemosynthesis in the Plant World [in Russian] (Sel'khozgiz, 1935).

[12] M. Moiseeva, Doklady Akad. Nauk SSSR 46, 3, 127 (1945).

[13] S. Ya. Sokolov, Bot. Zhur. 38, 5, 661 (1953).

[14] A. A. Tabenetskii, Izv. Akad. Nauk SSSR, ser. biol. 5, 609 (1947).

[15] A. A. Tabenetskii, Bot. Zhur. 37, 531 (1952).

[16] A. A. Tabenetskii, Izv. Akad. Nauk SSSR, ser. biol. 1, 71 (1953).

[17] K. A. Timiryazev, Selected Works on Chlorophyll and the Assimilation of Light by the Plant [in Russian] (Izd. AN SSSR, 1948).

[18] V. Kh. Tutayuk and YU. M. Agaev, Izv. Akad. Nauk AzerbSSR 5, 57 (1956) [in Azerbaidzhanian, resume in Russian].

[19] V. Kh. Tutayuk and Yu. M. Agaev. Theses Reports of the Second All-Union Conference on Photosynthesis [in Russian] (Izd. Moscow State University, 1957).

[20] E. I. Yanson, Theses Reports of the Second All-Union Conference on Photosynthesis [in Russian] (Izd. Moscow State University, 1957).

[21] D. F. Protsenko and L. K. Polishchuk, The Physiologycal and Biochemical Features of Frost Resistance of Fruit Crops [in Russian] (Izd. Kiev State University, 1948).

[22] C. Deneke, Über nicht assimilierende Chlorophylkörper. Inaug. Dissertation, Bonn (1880).

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ELECTRON-MICROSCOPE INVESTIGATION OF THE CHLOROPLASTS OF BELLIS PERENNIS IN THE SPRING

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In a previous work, we gave the results of electron-microscope investigations of the chloroplasts of the daisy in the autumn-winter period [1]. It was shown that in the summer chloroplasts have an oval form *, a porous structure, contain thickened parts, and are constituted from grana; osmiophyllic grana in the peripheral part of the chloroplast are absent. The characteristic features of the structure of the chloroplasts in the winter are the circular form, the dense structure, the many layers of the grana, and the abundance of osmiophyllic granules in the peripheral portion. The diameter of the chloroplasts in the winter is $^1/_4$ to $^1/_2$ of the size of the summer chloroplasts. It is also of interest to study the condition of the chloroplasts in the spring.

METHOD

Daisy plants overwintered in the open ground. At the end of March the leaves were completely free of snow, had a clear green color and good turgidity. In May, a massive dying of the overwintered leaves took place, completely ending toward the beginning of June. In the indicated period (March-May) samples were taken once a month. An investigation was made of chloroplasts isolated from the leaf blades. The preparations were prepared in the same manner as described earlier [1, 2]. A UEM-100 microscope was used. The light field method of investigation and the dark field method were used. Dark field pictures of the chloroplasts were obtained by shifting of the aperture diaphragm perpendicular to the optical axis, as a result of which the electrons moving through the object without diffusion were completely retarded. The dark-field and the corresponding light-field pictures of the chloroplasts were exposed on acceleration of the voltage, V = 60 kv.

RESULTS OF INVESTIGATIONS

A chloroplast from a daisy leaf in March is shown in the microphotographs, Fig. 1, a, b. The grains and osmiophyllic granules are clearly seen in the light-field (Fig. 1a) and dark-field (Fig. 1b) pictures. The grana are tightly arranged; on one projection, several partially overlapping grana can be separated. The osmiophyllic granules are very numerous both in the central and peripheral parts of the chloroplast. On the dark-field microphotograph we see that the peripheral part contains osmiophyllic granules and is devoid of grana. In Fig. 2, a, b, we see a chloroplast taken on the date as the sample which has, however, a somewhat different structure: the borders of the grana are not sharply expressed; on the dark field picture (Fig. 2 b) two layers can be differentiated in the peripheral part; the external layer possesses less consistency and, apparently, is a membrane of the chloroplast.

A chloroplast of the April fixation is shown in Fig. 3, a, b. In the dark-field picture (Fig. 3b) the osmiophyllic granules are seen more clearly than on the light field (Fig. 3a); the quantity of osmiophyllic granules is less than was observed in March. With the exception of the osmiophyllic granules, which are separated in view of the rough granularity, the distribution of the intensity (brightness) on the light-field picture is the same.

*We have in mind the outline of the chloroplast in a front view.

On the dark-field picture it is possible to separate, in the central part of the chloroplast with careful observation, the light and dark parts; there is no sharp border between these parts. The peripheral part is differentiated from the central part by the lower density.

In May the distribution of intensity on the light-field (Fig. 4a) and dark-field (Fig. 4b) pictures of the chloroplasts is the same. The osmiophyllic granules can be seen; their size and number is less in comparison to the data of the March and April observations.

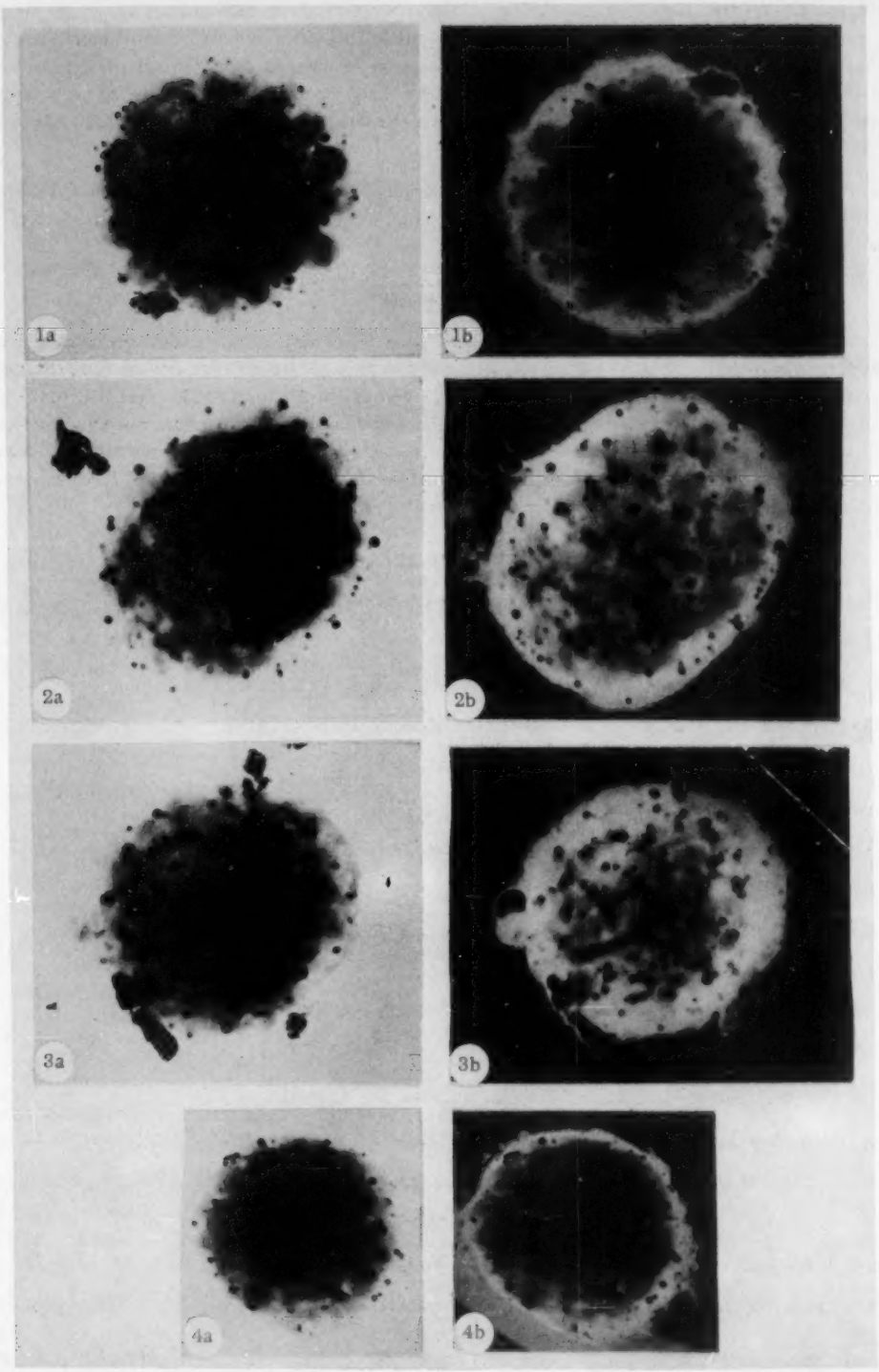
DISCUSSION OF THE RESULTS OBTAINED

In this work, an electron-microscope investigation of the chloroplasts of daisy in the light and dark field is presented. The necessity of the use in a parallel manner of the two methods is indicated by the characteristics of the object of investigation and the possibilities of these methods [3, 4]. There are thin and thick parts in the structure of the chloroplasts. From the data in the literature, it follows that it is expedient to use the dark-field method of investigation for these objects. In the light field the stream of electron passing through the object without diffusion forms a background which reduces the contrast range. With the dark-field method, the electrons passing through the object without diffusion are retarded by the aperture diaphragm. The contrast range in this case increases because it is determined only by the relationship of the quantity of electrons diffused by the parts of different thicknes.

When the material fixed in March was examined, it was found that the chloroplasts are somewhat different in structure. Some of these appeared quite transparent in relation to the electron beam and had clearly differing grana and osmiophyllic granules both in the light and dark fields. The light-field picture in this case gave additional information only about the structure of the peripheral part (see Fig. 1). On the light-field picture of other chloroplasts the grana were not visible and the osmiophyllic granules of the central part were poorly visible. The investigation of such a variety of chloroplasts on the dark field made it possible to show the grana and osmiophyllic granules of the central portion (see Fig. 2). Further observation of the chloroplasts in April and May showed that their changes are continuing the tendency toward a disappearance of the grana already noted in March. An increase in the contrast range by use of the dark-field method for the April sample made it possible to show the parts of different density on the corresponding microphotographs (see Fig. 3). Grana were not found in the structure of the chloroplasts fixed in May either by the light-field method of investigation or by the dark-field method (see Fig. 4).

The presence of grana is a characteristic feature of the structure of the chloroplasts of higher plants. The beginning of the modern idea on the granular structure of the chloroplasts was given in the work of Heitz [5]. Later, the same author [6], and also Duevel and Mevius [7] and Strugger [8] showed that the fluorescence of the chlorophyll in the chloroplasts is limited by the boundaries of the grana. The data from the electron-microscope investigation of Thomas and his colleagues [9] made possible the conclusion that chlorophyll is localized in the lamella of the grana. In a number of works it was shown that the fluorescence of the proplastids at first is the same, but the differentiation of stroma and grana takes place later [10, 11]. On this basis, Heitz and Maly consider that the chlorophyll at first is equally distributed in the stroma and the formation of the primary grana proceeds by the attainment in it of a certain concentration. The intimate connection between the quantity of chlorophyll and the structure of the plastids is attested to by the absence of grains and lamella in the plastids of the nonchlorophyll-containing leucoplasts of the etiolate leaves of chicory and their appearance in the latter when exposed to light [13]. The disappearance of grana in the chloroplasts of daisy in the spring in this connection is an indication of the decrease in the quantity of chlorophyll in them.

It is obvious that photosynthesis is resumed in daisy leaves after the plants are freed from under the snow, as similarly noted in hazelwort, which is similar to daisy in its biology. It is known that in the lighted leaf the continuous processes of synthesis and decomposition of chlorophyll leading to the restoration of the pigment take place. In recent years there have been a series of tests undertaken to put a quantitative value on the rate of restoration of chlorophyll in the leaf, although the data obtained cannot be considered conclusive at this time [15, 16, 17]. At the same time, according to the data of Vorob'eva and Krasnovskii [18], there is a bleaching of the photochemically unstable form of chlorophyll in the process of photosynthesis. The relationship between the resistance to light of the aggregate form of chlorophyll and the photochemically unstable monomeric form is determined by the conditions in which the plant is located. Thus, a decrease in the temperature of the



Chloroplasts of daisy leaves. 1, 2) March; 3) April; 4) May; a) light-field picture; b) dark-field picture (12,000 X).

surrounding environment causes an increase in the quantity of the aggregate form in the leaves of sugar beet*. With an increase in the temperature, the quantity of the monomeric form increases. Changes in the properties of chlorophyll under the effect of temperature show the possibility of the reversible and nonreversible transformations of the different forms of chlorophull for changes in the conditions of the external environment. Apparently, a decrease in the quantity of chlorophyll in the chloroplasts of the daisy in the spring is expalined by a distrubance in the condition of the mobile balance as a result of an inhibition of the process of synthesis or pigment in the old leaves.

The second feature of the change of chloroplasts of daisy in the spring period is the fact that the number and size of the osmiophyllic granules decrease. It is characteristic that the osmiophyllic granules of the peripheral portion of the chloroplast undergo change first.

SUMMARY

When looking at the general cycle of the changes of daisy chloroplasts, we must note the significance of these changes. Apparently, the extreme consolidation of the chloroplasts and the change in their surface characteristics, reflected in the enrichment of the osmiophyllic granules of the peripheral layer,is explained by the absence of photosynthesis during the winter period, which was shown by our experiments with daisy leaves (unpublished), and also by data in the literature [14]. The renewal of photosynthesis in the spring is connected with the disconsolidation of the structure of the chloroplasts. The complete disappearance of the grana in May can be considered a sign of the collapse of the chloroplasts, resulting from the age of the leaf.

LITERATURE CITED

[1] P. A. Genkel' and R. S. Morozova, Fiziol. Rastenii 4, 509 (1957).**

[2] R. S. Morozova, Fiziol. Rastenii 4, 484 (1957). **

[3] I. G. Stoyanova, Biofizika 1, 362 (1956).

[4] R. S. Morozova and E. M. Belavtseva, Biofizika 3, 265 (1958).

[5] E. Heitz, Planta 18, 616 (1932).

[6] E. Heitz, Planta, 26, 134 (1936).

[7] D. Duevel and W. Mevius, Naturwissenschaften 39, 23 (1952).

[8] S. Strugger, Ber. dtsch. Bot. Ges. 64, 69 (1951).

[9] J. B. Tomas, L. C. Post and N. Vertregt, Biochim. et Biophys. acta 13, 20 (1954).

[10] E. Heitz and R. Maly, Z. Naturforsch. 8, 243 (1953).

[11] D. Duvel, Protoplasma 44, 239 (1954).

[13] M. Lefort, C.R. Acad. sci. 245, 437 (1957).

[14] M. de Deken-Greusom, Biochim. et Biophys. acta 14, 203 (1954).

[15] P. A. Genkel' and L. S. Litvinov, Izv. Biol. N.-I. Inst. pri Permskom Univ. 7, 3 (1930).

[16] F. V. Turchin, M. A. Guminskaya and E. G. Plyshevskaya. Izv. Akad. Nauk SSSR, ser. biol. 6, 66 (1953).

[17] A. A. Shlyk and T. N. Godnev, Fiziol. Rastenii Agrokhimiya, Pochvovedenie, 50.***

[18] V. M. Kutyurin, Doklady Akad. Nauk SSSR 106, 2, 355 (1956).

[19] L. M. Vorob'eva and A. A. Krasnovskii, Biokhimiya 21, 26 (1956).

*The experiments were carried out with green solutions of chlorophyll consisting of, apparently, a suspension of chloroplasts and their fragments.

**See English translation.

***As in original—Publisher's note.

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THE POSSIBILITY OF DETERMINING THE DURATION AND TIME COURSE OF DORMANCY IN POTATO TUBERS BY MEASUREMENT OF TISSUE CONDUCTIVITY

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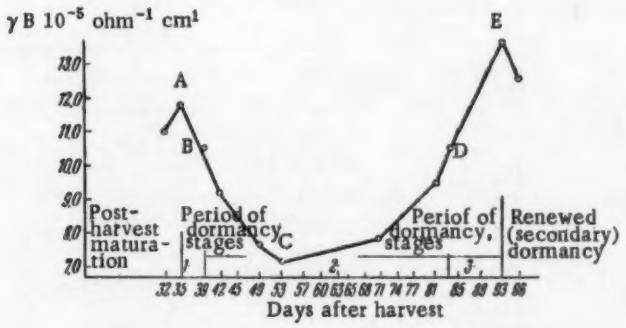
The demonstration of dormancy in potato tubers by means of external criteria is very inaccurate. Although microscopic analyses and observations of the isolation of cellular protoplasm [1] and of the transformations of reserve materials [2] throw light on the physical basis of dormancy and provide definite information on its depth, they are too laborious to perform.

In order to study the relative permeability of dormant woody tissues to electrolytes, Genkel'and Oknina [3] used the conductivity method. By measuring the conductivity of doubly distilled water into which electrolytes from tissue sections had passed by osmosis, Satarova [4] studied the relative permeability of potato tuber cells during the dormant period. Measurements made by us over several years have shown that the pattern of conductivity of small pieces of internal tissue from stored potatoes may be an index of the state of dormancy. It turned out that the data on conductivity patterns of dormancy are consistent with the observations of many years by potato growers.

Conductivity was determined by measurement of the resistance to a direct current or an audiofrequency alternating current by slices from potatoes of the same size collected from a single field at the same time. Resistance measurements were made at one temperature, at a current density of 0.44 milliamps per cm^2, using a conductivity bridge with carbon or zinc electrodes. With alternating current, capacitance was compensated. Because of the occurrence of drift, resistance was always measured at the same time (not less than 20 minutes) after application of the current to the tissue under the given experimental conditions.



Change in conductivity of the internal tissue of dormant Lorkh potatoes under cold storage. Carbon electrodes: current density, 0.44 milliamps per cm^2.

TABLE 1

Duration (in Days) of the Postharvest Maturation Period and the Dormant Period of Potatoes in Cold Storage, Established by the Tissue Conductivity Method

Variety	Postharvest maturation period	Dormant period	Inception of primary dormancy	Primary dormancy	Emergence from true dormancy
Ul'yanov	26	34	10	18	6
Épron	28	42	5	24	13
Lorkh	35	58	4	44	10
Mazhestik	38	62	0	48	14
Vol'tman	64	60	4	22	34

Results of measurements showed that the overall pattern of change of tissue conductivity during the dormant period with any given storage conditions and with any given tubers may be represented by a single curve. For example, this curve, shown in the figure, may represent the conductivity pattern of Lorkh potatoes under cold storage in Krasnodar. The curve shows maxima or minima at three points, the position of which is dependent on variety and also on storage and sprouting conditions—i.e., on basic factors determining the length of the dormant period and of its stages. Comparison of the conductivity pattern with the pattern of protoplasmic isolation established by the method of Genkel' and Oknina [1], and also with changes in water content, turgidity and starch content, revealed that these points (A, C, and E on the diagram) correspond to a definite change in these parameters.

If the division of the dormant period into three stages which has been taken by many workers [5] is adopted, point A, which marks the transition from the postharvest maturation period to the dormant period, should be considered the beginning of the first stage. This stage continues until the conductivity is reduced to the point at which it is impossible for the given variety of potato to sprout even under favorable conditions (point B in the figure). The decrease in conductivity which occurs in this stage of dormancy is accompanied by an increase in the degree of isolation of the cellular protoplasm. The starch content continues to increase, while moisture content remains practically the same.

TABLE 2

Tissue Conductivity of Dormant Potato Tubers in Cold Storage*

Variety	Minimal conductivity		Depth of primary dormancy, relative to the sprouting minimum ($\times 10^{-5}$ ohm^{-1} cm^{-5})	Depth of secondary dormancy, relative to emergence conductivity ($\times 10^{-5}$ ohm^{-1} cm^{-1})
	for sprouting	for the dormant period		
Ul'yanov	11.2	9.7	1.5	4.1
Épron	10.5	9.9	0.6	2.8
Lorkh	11.0	8.0	3.0	2.5
Mazhestik	11.3	10.1	1.5	5.0
Vol'tman	11.1	9.5	1.6	2.3

*Conductivity was determined with carbon electrodes at 22°. With other conditions conductivity values may be different.

TABLE 3

Criteria for Evaluation of Dormancy in Potatoes in Cold Storage in Krasnodar

Item evaluated	Criterion employed	
Earliness:	Duration of postharvest maturation period	
very early	< 20 days	
early	from 20 to 30 days	
average	from 30 to 40 days	
late	> 40 days	
Keeping quality:	Length:	
	of total dormancy	of the second stage
poor	to 30 days	to 15 days
average	from 30 to 40 days	from 15 to 30 days
good	> 40 days	> 30 days
Depth of primary dormancy:	Difference between minimal conductivities for germination and dormancy, $ohm^{-1} cm^{-2}$	
small	to 1.10^{-5}	
average	from 1.10^{-5} to $2.5 \cdot 10^{-5}$	
large	$> 2.5 \cdot 10^{-5}$	
Depth of secondary dormancy:		
small	to 2.10^{-5}	
average	from 2.10^{-5} to 3.10^{-5}	
large	$> 3.10^{-5}$	
Rate of transition to primary dormancy or emergence from it:	Length of corresponding stages:	
high	to 10 days	
average	from 10 to 20 days	
low	> 20 days	

During the second stage of dormancy, with the potatoes in cold storage, the moisture content and starch content of the tissues are almost unchanged. In this stage, cell protoplasm shows the greatest degree of isolation, and therefore it is only slightly permeable to electrolytes and exhibits less conductivity. During this time the conductivity of the tissue is always lower than that at which sprouting under favorable conditions is possible. Point C represents a critical time in the process of protoplasmic isolation and is the point of deepest dormancy. The actual conductivity value corresponding to this time is always higher in warm storage than in cold storage, which testifies to a less profound dormancy. Point B should be considered the beginning of the second stage of dormancy and point D the end of the state (see figure).

In the third stage of dormancy the conductivity requirements are less stringent for sprouting under favorable conditions. The beginning of this stage is marked by point D, and its termination by point E on the curve. During this phase the moisture content of the tissues is rising and the starch content is falling. In warm storage there is complete emergence from dormancy.

If during this period conductivity is greater than the minimal amount* required for sprouting, the buds begin to grow. In cold storage sprouting is not observed in most cases, since the conductivity is below the threshold level for sprouting. In such a case the tissue again becomes dormant, and the conductivity continues to in-

*The existence of a threshold conductivity for emergence from dormancy has been shown by other workers as well [4].

TABLE 4

Varietal Characteristics of Potato Tubers Determined on the Basis of Study of Conductivity Patterns of the Internal Tissues during Cold Storage

Variety	Characteristics of variety	
	according to conductivity data	according to data from IIKKh [6]
Ul'yanov	Early. Keeping quality average. Depth of primary dormancy average, of secondary dormancy, large. Becomes dormant with average speed and rapidly emerges from dormancy. Length of primary dormancy average.	Early, keeping quality undetermined
Épron	Early. Keeping quality better than average. Depth of primary dormancy small and of secondary dormancy average Becomes dormant rapidly and emerges from dormancy at average rate. Length of primary dormancy average.	Early, keeping quality good
Lorkh	Bears tubers in average time. Keeping quality good Depth of primary dormancy large and secondary dormancy average. Becomes dormant very rapidly and rapidly emerges from dormancy. Primary dormancy of long duration.	Bears tubers at average rate, keeping quality good
Mazhestik	Bears tubers in average time. Keeping quality good. Depth of primary dormancy average, of secondary dormancy large. In cold storage becomes dormant immediately upon completion of postharvest maturation. Emerges from dormancy at average rate. Primary dormancy of long duration.	Bears tubers at average rate, keeping quality good
Vol'tman	Late. Keeping quality good. Depth of primary dormancy small, of secondary, average. Becomes dormant very rapidly, emerges from dormancy slowly. Length of primary dormancy average.	Late

crease throughout this period. If the conductivity level, that is, the tissue permeability, is still too low for sprouting under the given storage conditions at the end of this period, the tissue once more becomes dormant, with the conductivity steadily increasing during passage through this stage. If, on the other hand, the conductivity becomes sufficient for sprouting under prevailing storage conditions, then sprouting occurs.

From this it follows that the distance between points A and E on the curve corresponds to the duration of the total dormant period, and the distance between points B and D to the duration of the period of true dormancy, i.e., the second stage. It is important to note that in warm storage, with a high enough conductivity, the swelling of buds and even the inception of sprouting may be observed during the postharvest maturation period and the first stage of dormancy. During the second stage, however, swelling and sprouting is impossible under any conditions. If these processes have already begun before the second stage is reached, they cease completely upon completion of the first stage and the buds may fall off. According to published information [2], this may be attributed to an insufficient supply of nutrients from the dormant tissue.

In order to demonstrate the feasibility of utilizing this method in evaluating the time course of dormancy, the conductivity of the tissues of potatoes of six well-known varieties which were grown in the field at the Krasnodar Potato Station was determined. With the data obtained from potatoes in cold storage, a table showing the duration of the dormant period and of its component stages, as well as that of the postharvest maturation period, was constructed (Table 1). In addition, the minimal conductivities occurring during the primary dormant period and the secondary dormant period were determined (Table 2).

The difference between the minimal conductivity necessary for sprouting under optimal conditions and the minimal value observed during the dormant period was taken as a measure of the depth of primary or secondary dormancy. We evaluated the keeping qualities of the tubers under given storage conditions according to the duration of primary and secondary dormancy. The experiment showed that tubers with a deep secondary dormancy may be safely stores at above-zero temperatures during the passage of the dormant period. A rapid passage into primary dormancy, as judged by the length of the first stage, and also prolonged emergence from dormancy, as judged by the length of the third stage, are indicative of good keeping quality. Tubers characterized by a shallow primary dormancy, a prolonged transition into dormancy, and a rapid emergence from it are more easily aroused from dormancy by external conditions. In addition, it turned out that the earliness of a given variety could be estimated according to the relative duration of the postharvest maturation.

In order to utilize conductivity data in evaluating the dormancy of a given variety, however, it is necessary to adopt certain criteria, which will be different for different storage conditions. The validity of such criteria may be checked by a comparison of characteristics of well-known varieties based on these criteria with those characteristics established by long-standing practice.

If the criteria listed in Table 3 are used to evaluate dormancy under our storage conditions, then it is possible to assign characteristics to known varieties on the basis of the data of Tables 1 and 2 which are in good agreement with the characteristics found by potato growers (Table 4). This method of characterization has the advantage that it contains quantitative evaluations and can be very precise.

SUMMARY

It is shown that the duration of the dormant state of potato tubers and the peculiarities of its time course can be indicated by studying the pattern of change of the electrical conductivity of the internal tissue.

LITERATURE CITED

[1] A. P. Genkel' and E. Z. Oknina, Diagnosis of Frost Resistance in Plants According to the Depth of Dormancy in Their Tissues and Cells [in Russian] (Izd. SSSR, 1954).

[2] S. M. Prokoshev, The Biochemistry of the Potato [in Russian] (Izd. AN SSSR, 1947).

[3] P. A. Genkel' and E. Z. Oknina, Inst. Fiziol. Rastenii Akad. Nauk SSSR 6, 1 (1948).

[4] N. A. Satarova, Doklady Akad. Nauk SSSR, 93 6, 1119 (1953).

[5] Selected Articles on the Potato [in Russian] Edited by N. Ya. Chmor and V. V. Arnautov (IIKKh, 1953).

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THE EFFECT OF SOIL PATHOGENICITY ON PHOTOSYNTHESIS AND CHLOROPHYLL CONTENT UPON COOLING OF CUCUMBER PLANTS

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Pathogenicity of cold soils [1, 2] is one of the important causes of death at above-zero temperatures of thermophilic vegetable plants. Under natural conditions, when there is a high moisture content and a comparatively extended cool period, this factor may determine the cold resistance of plants in the field, i.e., either death from infection of the root system occurs before the potential capacity to withstand cooling characteristic of the given plant is exhausted.

The occurrence of root rot during cold periods is a widespread phenomenon not only in thermophilic vegetable plants, cotton [3], and kidney beans [4], but also in such cold-resistant plants as clover [5-7], beet [8], tree seedlings [9], etc.

Species of Pythium, Rhizoctonia, Fusarium, and of other fungi have been reported as the causative agents. In nursery culture, an effective way of combatting root rot is to introduce fungicides into the soil in the vicinity of the roots, a method which increases the field resistance of plants to cold.

This method requires extensive field testing and also examination of physiological responses. Investigations using this method enable one to study separately the effect of soil pathogenicity and of cold on the plant.

The root system is, of course, in primary contact with the pathogenic organisms, and even in the early stages of infection it is stimulated to greater activity than is normally the case [2]. Investigations of this aspect should undoubtedly be continued. In 1958, in the course of experiments to clarify the effect on cucumber yield of introduction of a fungicide into peat-humus blocks, we investigated changes in photosynthesis and chlorophyll content occurring during cooling of plants.

Cucumber plants of varieties Murom and Nezhin were transplanted on May 19 to small pots containing peaty compost, some of which also contained thiuram (0.5 of a 50% dust of DMTD per kg of mixture). They were grown in a greenhouse without supplementary illumination. Four days before the next transplanting, the plants, which were in the third-leaf stage, were transferred to a small growing house, and subsequently transplanted to small beds on June 3. Later some of the plants to be used in the experiments described below were dug up and transferred to thinning boxes. After the plants had recovered from transplantation, they were transferred to a cold chamber kept at 10-12° with artificial illumination (4000 lux) and a relative humidity of 80%.

The Murom cucumber were in the stage of profuse flowering, which follows close on the heels of the fourth leaf stage, and the Nezhin cucumber were in the fifth-sixth leaf stage, when flowering is beginning. In the other experiments, plants were grown in thinning boxes in the greenhouse.

Determination of photosynthetic rates was carried out before cooling and after a definite time in the cold chamber; in addition, measurements were made after portions of leaves of previously cooled plants had been kept in a damp chamber at 20° for 48 hours. Photosynthesis was measured in the Warburg apparatus using buffer No. 11 and a light intensity of 18,000 lux. No fewer than three replicates were used. Respiration was measured on the same tissues (10.17 cm^2) before exposure to light and for 30 minutes after exposure. At the same time, chlorophyll content was measured using 13 leaf discs 7.5 mm in diameter (two replicates). Determination of

TABLE 1

Reduction of Rate of Photosynthesis of Cucumber Plants Which Had Been Cooled on Ordinary (c) and Sterile (e) Soil (in mg CO_2 per in^2 per hr.)

Variety	Length of cooling period, in days	Immediately after cooling		After 48 hours warmth		Chlorophyll content, mg/g	
		c	e	c	e	c	e
Nezhin	9	14.73	25.15	15.31	25.10	1.42	2.34
Murom (4th leaf)	9	15.38	21.61	8.18	14.81	0.29	0.53

chlorophyll in leaves which had been in a damp chamber 48 hours was made using the same material which had served for measurements of photosynthesis. A sample (130-160 mg) was ground with sand and the chlorophyll extracted with 96% ethyl alcohol (10 ml) with suction through a glass filter. Chlorophyll content was determined colorimetrically. A calibration curve was constructed using a solution of crystalline chlorophyll obtained from O. P. Osipova, to whom we are indebted.

Results of the first orienting experiments, in which the rate of photosynthesis of plants cooled at 10 deg under artificial light on ordinary (c) and sterile (e) soil was measured, are presented in Table 1.

In this experiment Nezhin cucumbers were grown on soil sterilized in the autoclave and Murom cucumbers on soil to which thiuram had been added before sowing (0.5 g per kg of soil).

The data of Table 1 show that under conditions in which the effect of soil pathogenicity is excluded the rate of photosynthesis is reduced to a small extent.

The pattern of change in photosynthesis as induced by different degrees of cooling was studied in greater detail with the two varieties. The third leaf was used. Data from these experiments fully support the conclusion made above that there is a greater reduction of photosynthesis when plants are cooled in the presence of a soil microflora (Table 2). This becomes especially clear upon examination of the second column, which shows the reduction of photosynthesis rate from the original value (before cooling) in percent.

Since Murom cucumbers were more sensitive to cold, the experiments with them (Table 2) illustrated more strikingly the fact that with transfer of plants which had been cooled 6-11 days to warm conditions there is a further reduction in rate of photosynthesis which is greater for plants which had been cooled on soil containing pathogenic microorganisms.

TABLE 2

Photosynthesis in Cucumber Plants Which Had Been Cooled on Ordinary (c) Soil and on Soil to Which Thiuram (e) Had Been Added (in mg CO_2 per in^2 per hr)

Treatment		Murom			Nezhin		
		CO_2 in mg per in^2 per hr	reduction in % of original	increase with increase of light intensity from 9.5 to 18 thous. lux	CO_2 in mg per in^2 per hr	reduction in % of original	increase with increase of light intensity from 9.5 to 18 thous. lux
Before cooling	c	28.94	—	—	25.75	—	70.5
	e	27.13	—	—	27.87	—	73.8
Two days in warmth	c	23.93	17.3	80.9	24.31	5.6	92.0
	e	23.72	12.6	79.7	28.03	0	99.0
Six days of cold	c	23.40	19.1	120.7	20.22	21.5	75.2
	e	25.18	7.2	85.1	23.50	16.7	81.3
The same + two days in warmth	c	11.86	59.0	106.6	19.67	23.6	104.9
	e	19.24	29.1	106.6	21.85	21.7	131.7
11 days of cold	c	12.77	55.9	85.1	2.71	89.5	15.8
	e	15.06	44.5	34.2	10.02	64.9	39.0
The same + two days of warmth	c	2.59	91.1	68.1	0.32	—	—
	e	9.58	64.7	17.8	12.62	—	—

TABLE 3

Effect of Cooling of Isolated Leaves of Vyaznikov Cucumber on Their Chlorophyll Content (in mg per g dry wt.)

Treatment	Content, mg	Destroyed	
		mg	%
Before cooling	2.34	-	-
Two days of cold	2.30	0.04	1.7
The same + one day of warmth	1.60	0.74	31.6
Three days of cold	2.31	0.03	1.3
The same + one day of warmth	1.54	0.80	34.2
Six days of cold	2.05	0.29	12.4

It can be seen in Table 2 that after a six-day cooling period, control plants suffered a further reduction in photosynthetic rate upon return to warmth of 39.9% (from 19.1% to 59%), while experimental plants showed a further reduction of 21.9% (from 7.2% to 29.1%). The same picture is observed in the case of an 11-day cooling period. These differences are seen still more clearly if the reduction which occurs after previously cooled plants have been kept in a warm place is expressed in percent of the rate observed immediately after cooling. Reductions in rate of photosynthesis occurring during a two-day period in warmth are as follows. After 6 days' cooling on ordinary soil, there is a 49.3% reduction, and on treated soil it is only 23.6%. After 11 days' cooling it is 79.7% on ordinary soil and 36.4% on treated soil. In the experiment with the fourth leaf (Table 1), after a 9-day cooling period photosynthesis in the control plants was reduced 46.8% and in the treated plants 31.5%. The same patterns were observed with Nezhin cucumbers (Table 2), but they were less marked; this is probably associated with the fact that this variety has a greater resistance to 10-12* temperatures.

TABLE 4

Chlorophyll Destruction in Cucumber Plants Which Were Cooled on Ordinary (c) and on Thiuram-Treated (e) Soil (in mg per g dry wt.)

Treatment	Murom			Nezhin		
	content, mg	destroyed		content, mg	destroyed	
		mg	%		mg	%
Before cooling (c)	1.43	—	—	1.13	—	—
Before cooling (e)	1.42	—	—	1.20	—	—
Two days in warmth (c)	0.97	0.46	32.2	0.90	0.23	20.4
Two days in warmth (e)	1.23	0.19	13.4	1.13	0.0	0
Six days in cold (c)	0.83	0.60	42.0	1.03	0.10	8.8
Six days in cold (e)	1.20	0.22	15.5	1.16	0.04	3.3
The same + two days in warmth (c)	0.63	0.80	56.0	0.59	0.54	49.6
The same + two days in warmth (e)	1.09	0.33	23.2	0.75	0.45	37.5
11 days in cold* (c)	0.52	0.91	63.6	0.48	0.65	57.5
11 days in cold* (e)	0.77	0.65	45.8	0.92	0.28	23.3
The same + two days in warmth (c)	0.22	1.21	84.6	0.27	0.86	76.1
The same + two days in warmth (e)	0.60	0.82	57.7	0.72	0.48	40.0

*Nezhin cucumbers were cooled 12 days.

TABLE 5

Aftereffect of Cooling of Etiolated Cotyledons on Chlorophyll Formation (in Warmth) (in mg per g dry wt.)

Expt'l. material	Method of evaluation	Length of cooling period (in days)						
		0	2	3	4	5	6	7
Nezhin cucumbers	in mg	1.18	—	0.74	—	—	0.60	0.46
	in %	100	—	62.7	—	—	50.8	39.0
Vyaznikov cucumbers	in mg	0.06	0.88	—	0.84	—	0.66	0.61
	in %	100	83.0	—	79.2	—	62.3	57.5
Kolkhoz melons	in mg	0.70	0.53	—	—	0.33	0.28	—
	in %	100	76.4	—	—	47.4	40.0	—

One of the most important causes of the reduction of photosynthesis in thermophilic plants which results from cooling may be chlorophyll destruction, especially when the cold period is long. Under natural conditions a certain amount of fading is always noted and there is even a tendency toward yellowing the "sick" plants. This is of interest in connection with the possibility of diagnosing the degree of cold resistance of a plant in accordance with the rate of chlorophyll destruction during or after cooling.

Results of an experiment in which separate leaves of Vyaznikov cucumbers were cooled at 3° are presented in Table 3. They show that in this variety there is no significant destruction of chlorophyll during the first three three days of cooling, but that destruction occurs after previously chilled leaves have been in warmth one day. Cooling of individual leaves of a more resistant plant, Novocherkassk pepper, at 3-8°, resulted after 10 days in a 22.1% destruction of chlorophyll (the same period + two days in warmth—30.5%), and after 13 days in a 31.7% destruction.

A more detailed analysis of chlorophyll content in the leaves of plants grown and cooled on ordinary and thiuram-treated soil was made in the above-described experiments to determine photosynthesis rates in Murom and Nezhin cucumbers.

The data of Table 4 show first of all that there is almost no chlorophyll destruction in Nezhin cucumbers exposed to a six-day cooling period at 10-12° under artificial illumination. The plastid apparatus of Murom cucumbers is less resistant and with the same treatment there is a more intensive chlorophyll destruction. The data indicate, second, that there is a significant amount of destruction in previously chilled plants during exposure to conditions of warmth. Finally, Table 4 shows that exclusion of the factor of soil pathogenicity slows up chlorophyll destruction occurring both during the cooling period and during the following warm period.

With a more cold-resistant plant, pepper, we also observed a more rapid destruction of chlorophyll on untreated soil. In an experiment previously described [2], cooling at 7-8° for 12 days of flowering Novocherkassk pepper plants resulted a chlorophyll desturction during a subsequent 24-hour warm period of 23% on untreated soil and 15.3% on treated soil.

Studies of recent years [15] have shown that in addition to being an absorptive organ the root is the site of synthesis of substances required by the plant. Material obtained in these studies shows that pathogenic microorganisms in the soil, by infecting the root system, disrupt its metabolism. This is reflected in injury to the plant's chlorophyll apparatus, in reduction of the photosynthetic rate, and in heightened destruction of chlorophyll.

A comparison of data on photosynthesis (Table 2) and on chlorophyll (Table 4) reveals that the reduction in photosynthesis is often proportional to the rate of chlorophyll destruction. In Murom cucumbers, however, there was a 42% chlorophyll destruction and a 19.1% reduction in photosynthetic activity after a six-day cooling period. It is of interest that these leaves react more sharply to a change in light intensity (Table 2). For example, if the plants are exposed to a low light intensity (9.5 thousand lux) and it is then increased to 18 thousand lux, i.e., by 89%, those cooled on ordinary soil show an increase in photosynthetic rate of 120.7%, while those cooled on treated soil, i.e., the less injured plants whow an increase of 85.1%, which is nearly proportional to the increase in light intensity. After a period in warmth, plants in both groups still react to a change in light intensity more sharply than is usual.

The same pattern was found with Nezhin cucumbers, but somewhat later; plants cooled for six days and subsequently exposed to warmth showed a 49.6% chlorophyll destruction for the control group and a 37.5% destruction for the experimental group. With an increase in light intensity, the rate of photosynthesis in the control plants increased 104.9% and in the treated plants 131.7%. In other cases the response to a change in light intensity was within normal limits, i.e., approximately proportional to the increase in light intensity.

As Kursanov and Kryukova [13] observed, the heightened photosynthetic activity occurring at the begining of chlorophyll destruction may be related to the accumulation of degradation products. This capacity is soon lost, however, with the continuing degenerative changes in the plastids which accompany further destruction of chloropyll.

The rate of photosynthesis is one of the factors involved in the relation of photosynthesis to plant productivity [14]. A small reduction in photosynthetic activity during cooling of plants grown on soil to which a fungicide had been added was naturally expressed, therefore, in greater productivity.

A. K. Solov'ev performed an experiment to determine the effect on cucumber yield of introducing thiuram into small pots containing peaty compost. The plants were transplanted to plots on June 3. The summer of 1958 was characterized by an excessively large number of cool days. Each treatment group comprised 25 plants. On August 27, the average fruit yield per plant in the Murom cucumber was 271 g for the control group and 403.9 g for the experimental group, i.e., treatment of the soil with thiuram increased the yield 49%; in Nezhin cucumber the increase was 47.8%. These data, and also those obtained from physiological studies, thus indicate the necessity of extensively testing this method of controlling pathogenicity in cold soils.

The injurious efect of cold on thermophilic plants may be judged not only in terms of the degree of chlorophyll destruction, but also by the inhibition of its formation [11, 12]. We have designed the following experiments to determine this. Etiolated seedlings were grown for three-four days on sand or filter paper at 26-30°. Unopened cotyledons were removed, laid out in Petri dishes on moist filter paper and transferred to a chamber kept at 5-8°. The uncooled sample was kept in diffuse light in the laboratory for four days; chlorophyll was then extracted from whole cotyledons or portions of them (200-300 mg) with 10 ml of 96% ethyl alcohol and determined colorimetrically; the experiment was replicated twice.

The data presented in Table 5 show that chlorophyll formation is extremely sensitive to cold. An effect is noticeable even after two-three days' cooling. In Nezhin cucumbers, chlorophyll formation had been reduced by almost half after a six-day cooling period, while chlorophyll destruction in the leaves during this same period had only begun (Table 4).

Thus, the process of chlorophyll formation in the cotyledons is more sensitive to cold than that of chlorophyll destruction in the leaves and probably it could be used to diagnose the degree of cold resistance of thermophilic plants.

We attempted to isolate the organism responsible for the pathogenicity of cold soils in our experiments. Nerosim and Vyaznikov cucumber plants grown on untreated soil were cooled for five days at 6-7° in the cotyledon stage. A section of tissue 5 cm long taken from the region of the root crown and including some root tissue and some hypocotyl tissue was sterilized for 5 minutes with 10% H_2O_2; the peroxide was washed away with sterile water and the tissue sections were then placed in Petri dishes on Czapek's agar medium or an oat infusion; the Petri dishes were kept at 25°. After two-three days colonies of fungi began to form on the tissue sections and to grow rapidly until a dense layer of white mycelium was produced.

The same fungus was always isolated. Its mycelium is syncytial, branching, and filled with a homogeneous, finely granular protoplasm. Protoplasmic movement was often observed in the thick central hyphae, particularly with long periods illumination; the sporangia are large and spherical and located singly at the ends of hyphae, sometimes appearing like sac-like swellings of the hyphal tips. It is not always possible to transfer the mycelium to fresh Czapek's medium, but, where this has been done the fungus grows vigorously for two-three days. Parts of chilled plants which have been sterilized with H_2O_2 and placed in tap water become covered with a dense mycelium on the fourth or fifth day.

The isolated fungus was identified by A. E. Protsenko, a co-worker in the Institute of Microbiology of the USSR Academy of Sciences, to whom we are indebted. The fungus turned out to be Pythium debaryanum Hesse. Isolated strains of the fungus infected healthy plants if the mycelium or infected plant tissue was inoculated in a drop of water into the tissue of a leaf axil. In a damp chamber at 20-25°, the leaf petiole shows signs of

infiltration on the second or third day; the mycelium then spreads up and down along the stem and the plant dies. Pythium, which is always present in the soil, almost never infects cucumber seedlings at temperatures optimal for the plant. This is probably because its growth at these temperatures is inhibited by microbial antagonists. The suspicion thus arises that one of the reasons for the high pathogenicity of cold soils is the relatively greater suppression by low temperatures of the activity of biological antagonists of the pathogenic organisms.

SUMMARY

Reduction of the rate of photosynthesis as a result of prolonged cooling of the plant and subsequent cultivation in the warmth is smaller under conditions in which the action of pathogenic microorganisms is excluded, e.g., when a fungicide is introduced into the soil in the vicinity of the roots.

Destruction of chlorophyll is one of the main causes of reduction of the photosynthetic rate in plants after prolonged cooling, especially when the plants are subsequently transferred to warmth. Pathogenicity of the soil accelerates destruction of chlorophyll.

Formation of chlorophyll is very sensitive to previous cooling. The aftereffect of cooling on chlorophyll formation and destruction may be employed as an index of the cold resistance of thermophilic plants.

Pythium debaryanum Hesse is the organism responsible for pathogenicity of cold soils on which cucumbers are cultivated.

LITERATURE CITED

[1] L. A. Nezgovorov and A. K. Solov'ev, Fiziol. Rastenii, 4, 489 (1957).

[2] L. A. Nezgovorov and A. K. Solov'ev, Fiziol. Rastenii 5, 424 (1958).

[3] D. B. Aslanov, Inst. Zemledeliya Akad. Nauk Turkm SSR 1, 89 (1957).

[4] P. Chatterjee, Phytopathology 48, 197 (1958).

[5] A. A. Meier and N. I. Krivodubskaya, Selekts.i semenov. 8-9, 66 (1937).

[6] N. N. Lavrov, Trudy Tomskogo Univ. 117, 143 (1952).

[7] L. S. Milovidova, Trudy Tomskogo Univ. 141, 103 (1957).

[8] D. L. Tverskoi, Agrobiologiya 2, 123 (1949).

[9] E. Wright, Phytopathology 47, 658 (1957).

[10] L. K. Polishchuk, Physiological Processes Occurring in Pumpkin at Reduced Temperatures [in Russian] (Izd. Kievskogo Univ., 1940).

[11] L. K. Polishchuk, Doklady Akad. Nauk SSSR, 70, 3, 529 (1950).

[12] S. S. Andreenko and Z. V. Titova, Doklady Akad. Nauk SSSR, 116, 1, 157 (1957).

[13] A. L. Kursanov and N. N. Kryukova, Biokhimiya, 22, 391 (1957).

[14] A. A. Nichiporovich, Photosynthesis and the Theory of Obtaining High Yields. Timiryazev Lecture. XV [in Russian] (Izd. AN SSSR, 1956).

[15] A. L. Kursanov, The Interrelationship of Physiological Processes in the Plant. Timiryazev Lecture, XX [in Russian] (Izd. AN SSSR, 1959).

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SOME PECULIARITIES OF SHOOT FORMATION IN VYATKA WINTER RYE IN WHICH THE VERNALIZATION STAGE IS TERMINATED AT A HIGH TEMPERATURE

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Studies of the dynamics of shoot formation in various cerials have been made by many workers. Some have studied it in relation to the level of mineral nutrition [1-4], while others have studied it from the point of view of the effect of altering the conditions under which the plant develops so that the normal relations between growth and development rates are disturbed. Studies concerned with the effect of more than one factor at the same time are unknown to us.

These investigations convincingly show that it is possible to induce a significant increase in the number of tiller shoots, of spikelets in a spike, of flowers in a spikelet, and of other plant organs either by increasing the nutritional level or by retarding a given stage of development under conditions favorable to growth. In such investigations, however, only more or less developed shoots which are microscopially visible were counted. Microscopic analysis shows that in the neighborhood of the meristems there are a large number of primordia of various organs which for some reason have not developed further. These primordia represent the plant's reserve potential, and therefore deserve attention.

It is often observed experimentally that a given factor may bring about a marked change in the number of shoots laid down, but only a certain number of these, which is characteristic for the plant under ordinary conditions, develops normally. If the experimenter does not take this into account, the significance of the factor under consideration may not be realized.

A count of all the organ primordia laid down may be suitable in selection practice as well, since it provides information on the variety's potential for increased productivity.

With regard to the increase in shoot number as a result of a retarded rate of passage of a given stage of development, investigators explain it as being due simply to the prolongation of organogenesis characteristic of this stage. It is well known that under such conditions there are substantial changes in the organism's hereditary properties; it becomes more plastic with an increased vitality.

Material obtained experimentally in the studies under discussion does not permit a decision as to whether the increase in number of shoots formed is only a result of an increase in the duration of the period of formation, or whether during this prolonged period there is also a change in the organism's hereditary properties.

This study is an attempt to work out some of the problems involved.

EXPERIMENTAL METHODS AND DESIGN

Experiments were performed at the biological station of the A. I. Gertsen Leningrad State Pedagogical Institute in 1953-1954. Plants were grown in clay pots containing 5.3 kg of absolutely dry soil. Ten pots were used for each treatment, each containing 5 plants after thinning.

TABLE

Effect of Nutritional Conditions and Term of Vernalization on the Number of Tiller Shoots, Spikelets and Flowers Laid down in Vyatka Rye

Treatment	No. of days of vernalization	No. of tiller shoots		No. of spikelets in a head		% of heads with maximal no of flowers in a spikelet				
		average	maximal	average	maximal	5	6	7	8	9
Control	40	6.8	14	46.8	50	5.3	31.8	42.4	5.3	0
	50	4.2	7	35.5	43	15	70	15	0	0
Application at the third-leaf stage	40	12.8	31	50.1	58	0	14	52.2	29	4.8
	50	7.5	13	35.5	40	5	25	60	10	0
Application at the tillering stage	40	16	42	59.1	62	0	18.2	22.7	31.8	27.3
	50	9.2	15	38.6	45	0	0	55.5	44.5	0
Removal of tiller shoots	50			40.7	44	0	0	50	50	0

Note: 1) In one treatment in which plants were supplied abundantly with mineral fertilizers, leaves exhibited burns; no positive results were obtained. 2) In all treatments, with the exception of the control, plants were abundantly supplied with mineral nutrients.

Results of an analysis of the soil used are:

K 20.0 mg, P 7,5 mg per 100 g air-dry soil, N (total) 0.164% of the absolute dry weight of the soil, pH 5.27 (aqueous suspension). The lime requirement was determined by Skorik's method [5].

The optimal ratio of mineral elements in the fertilizer was determined in a series of preliminary experiments. The amount of fertilizer to be given was determined in an exploratory experiment carried out under the same conditions as the main experiment but 10 days earlier. In essence this experiment consisted of the application of different doses of fertilizer containing the various minerals in the ratio previously shown to be optimal The dosage level which was shown to give the maximal plant growth was selected; the slightest increase in this level resulted in a decrease in growth. Considering that the exploratory experiment ran for 10 days prior to the main experiment, plants were exposed to the dosage level selected during this period and during the main experiment.

The number of spikelets and flowers in a spikelet which were laid down was counted with an MBS-2 stereoscopic microscope. Counts were made at the time of the appearance of the seventh stem leaf, since at this time the formation of new primordia was beginning to stop, while the growing points still retained their form (had not shriveled) and were not yet covered with bracts; the counts were easier to make at this time for this reason. In plants which had been vernalized for 50 days, the heads of the three most strongly developed shoots on each plant were examined, making a total of 150 shoots. In the incompletely vernalized plants, all shoots which had reached the proper stage of development were examined, the total number being approximately 150.

Seeds were vernalized in a ZIL electric refrigerator at 2°. One group of seeds was vernalized for 50 days, and the other for 40 days. Four treatment groups were established. In the first, the control group, no fertilizer was applied, and no lime was applied. In the remaining groups, 0.5 of a dose of N + 1 dose of PK was given before sowing, and 5 doses of NPK were given in two applications after sowing; these amounts are those determined to be optimal in the exploratory experiment. In the third group, the first application after sowing was made in the tillering period, and in the second and fourth groups it was made just prior to the appearance of the third leaf. In all groups the second application was made at the time the floral primordia were being laid down. In addition, lime was added to the soil before sowing on the basis of 1.1 g per kg of soil, and microelements were also added in small amounts at this time.

One dose of NPK contained 0.6 g of NH_4NO_3; 0.175 g NaH_2PO_4 and 0.236 g K Cl.

MORPHOLOGICAL OBSERVATIONS

Seeds were sown on May 20. Shoots appeared on May 23. The third leaf appeared on May 31. Tillering began on June 2. This stage and all the previous stages were reached at the same time. Succeeding stages were reached at different times depending on the length of the vernalization period. Inception of shoot elongation occurred on June 18 in plants vernalized 50 days and on June 27 in plants vernalized 40 days. In certain plants in this group, shoot elongation began as late as the day the experiment was terminated.

Since the number of spikelet and flower primordia was determined at the beginning of the heading stage, this stage and also the following stages, could not be observed. With a few pots especially set aside for this purpose it was established,however thatin plants of the first group heading began on June 30, with little variation among shoots; flowering began on July 12.

In the second group, certain individuals began to head on July 4. Heading, and also shoot elongation, did not occur readily, as could be predicted from the fact that in each tuft there were heading shoots, shoots in various stages of elongation, and shoots without visible signs of internode lengthening. In these plants flowering began on July 17. After this the experiment was taken down.

From the time course of development as well as the nature of the developmental stages observed, it may be concluded that the low temperature requirement of Vyatka rye is completely satisfied by a 50-day vernalization period under the conditions of our experiment; with a 40-day vernalization period, the plant completes its vernalization stage after sowing. Systematic observations of the air temperature show that it was below + 6° for only a few hours after sowing. Therefore, the completion of the vernalization stage in these plants took place for the most part at temperatures above normal.

EXPERIMENTAL RESULTS

Quantitative data are presented in Table 1 which show that the number of tiller shoots, spikelets in a head and flowers in a spikelet depends to a large extent on the nutritional level and on the length of the presowing vernalization period.

With an abundant nutrition, the number of tiller shoots (average) was 2.1 times as great as that of the control. Approximately the same value (2.3 times) is obtained in incompletely vernalized plants. If, however, the maximal values are compared, it turns out that the effect of fertilizer on incompletely vernalized plants differs from that on vernalized plants: in the first case, treated plants have three times as many tiller shoots as control plants, and in the second, only twice as many.

In order to express quantitatively the effect of completion of the vernalization stage at a higher temperature on tillering activity under a given nutritional regime, we calculated the ratio of the number of tiller shoots in plants vernalized 40 days prior to sowing to the number in plants vernalized 50 days. It was shown that under these conditions the number of shoots may be increased by 158.1-177%. The combined effect of incomplete vernalization and abundant nutrition on tillering activity was to increase the number of shoots to a value 3.72 times that of the control.

The pattern of dependence of the number of spikelets in a head on vernalization procedures shows (Table 1) that in plants vernalized 50 days prior to sowing this is an extremely stable character. This is apparently due to the reinforcement of the conservatism of heredity associated with passage through the vernalization stage. Even if the tiller shoots are removed, a procedure which is effective in inducing the formation of heads [6,7], the number of spikelets laid down is increased only 14.6%. In incompletely vernalized plants an increase in the nutritional level had a somewhat greater effect: in plants supplied with nutrient fertilizer during the tillering stage, 26% more spikelets were laid down than in control plants. If the data for these plants is compared with the control for a 50-day vernalization period, there is a 66.7% increase. In sum, the number of spikelets in a head is increased to a markedly greater extent when vernalization is completed at a higher temperature than when there is merely an increase in nutritional level.

Are the conditions under which vernalization occurs manifested in the same manner by the number of spikelets laid down? In our work [8] it was shown that spikelets begin to be laid down after completion of vernalization. The first spikelet primordium appears above the last (according to formation succession) leaf sheath. Subsequently they appear in the axil of each primordial leaf, these occurring in basipetal succession. At the

same time, although at a slower rate, the spikelets are laid down acropetally. The leaf sheath are laid down during the vernalization period, and therefore the number of sheaths is increased with an increase in the duration of the period. In this way, the direct influence of a prolonged vernalization period is also manifested here. Evidently, however, morphological changes in the growing points cannot be ascribed to this influence alone. The increased number of leaf primordia on a meristem only creates a possibility of an increase in the number of spikelet primordia. The realization of this possibility, however, depends on the entire course of events of morphogenesis occurring in the next stage, the light stage.

In our experiment the light stage was completed under natural conditions and was, therefore, of normal duration for this variety of rye, as may be judged by results of the morphological observations. It must be assumed in this connection that the period of formation of spikelet primordia was also of normal length. It is obvious, however, that in incompletely vernalized plants this was more rapid than in completely vernalized plants. In our view this indicates a more vigorous physiological activity associated with morphogenesis. Therefore the increased number of spikelets in a head in plants which completed the vernalization stage at a higher temperature is due in large degree to changes in the metabolic pattern.

The capacity of a plant to form a given number of flowers in a spikelet was also found to depend both on nutritional level and on vernalization conditions.

Let us turn once more to Table 1. Among control plants, which were completely vernalized at a low temperature, the maximum number of flowers in a spikelet does not exceed 7. The majority of plants (85%) have not more than 6 flowers laid down. An increase in nutritional level shifted the maximum to 8. Of plants in the third treatment group, 44.5% had 8 flowers per spikelet. But in no case were more than 8 flowers per spikelet observed in this group. This was the limit which was not exceeded even with removal of tiller shoots, a procedure which enhanced flow of nutrients to the head.

In plants which completed the vernalization stage at an increased temperature but which received no fertilizer (control) 8 flowers per spikelet were laid down. With an improved level of nutrition these plants produced a maximum of 12 flowers per spikelet. At the same time, however, some of the plants produced no more than 6 flowers. The largest number of heads with spikelets containing 9 or more flowers was observed in the third treatment group. It should be pointed out that a significant number of branched heads was observed in this group.

The material presented shows that the number of flowers in a spikelet, and also the number of tiller shoots and of spikelets, may be greatly increased by completion of the vernalization stage at an increased temperature. But while the increase in the number of spikelets and tiller shoots can be explained in terms of a retarded rate of passage through the vernalization stage, this explanation cannot be extended to cover the formation of flower primordia, which occurs after vernalization, since the stages following vernalization are of reduced duration. Observations on late sowing of this variety using seeds vernalized 50 days gave special confirmation of this. Plants from this sowing formed the usual number (7-8) of flower primordia under favorable conditions of nutrient supply, although formation occurred at the same time as in incompletely vernalized plants in the growth experiment—the end of July and beginning of August. Therefore, the increased number of flowers, and all the more of branched heads, in plants which had completed vernalization at a higher temperature can only be explained by the greater plasticity of these plants, which was manifested when there was an abundant supply of mineral nutrients. It is fully understood that such a radical change in the nature of these plants has its beginning at the time of exposure to increased temperatures at the end of the vernalization stage. It can, therefore, be assumed that even the increase in the number of tiller shoots is due not only to an increase in the length of the tillering stage, but also to changes in the nature of the plant.

SUMMARY

1. The number of tiller shoots, spikelets and flowers formed in plants which have been vernalized at an optimal temperature is not changed as a result of an increase in the nutritional level to an extent proportional to the increase; this is because of the conservatism of plants so treated.

2. Plants which have completed vernalization at an increased temperature lay down significantly more tiller shoots, spikelets and flowers. Some of them form branched heads. The increase in number of tiller shoots and spikelets in these plants is due both to the prolongation of the vernalization period and to the increased plasticity of the organism. The increase in number of flowers in a spikelet is due solely to the increased plasticity of plants which have matured under conditions of abundant nutrient supply.

3. Growth of plants which have completed vernalization at an increased temperature under conditions of abundant nutrient supply may be utilized in selection practice as a method of obtaining forms from which high-yielding varieties can be developed, in particular, forms with branched heads.

This study was completed under the direction of Professor F. D. Skazkin.

LITERATURE CITED

[1] S. A. Alekperov, The Dynamics of Formation of Heads in the Light Stage [in Russian] (Baku, 1939).

[2] V. T. Eremenko and G. Ya. Sopatov, Nauchn. zap. Ukr. n. i. Inst. Sotszeml. 1, 2 (1940).

[3] D. A. Sabinin, Byull. Moskov. Obshch. Ispyt. Prirody, Otdel Biol., 44, 1 (1937).

[4] N. Z. Stankov, Doklady VASKhNIL, 13 (1939).

[5] I. L. Skorik, Doklady VASKhNIL 13, 14 (1938).

[6] T. D. Lysenko, Agrobiology [in Russian] (Sel'khozgiz, 1952).

[7] M. S. Miller, Doklady Akad. Nauk SSSR, 67, 6 (1949).

[8] V. A. Surkov, Development and Branching of Heads in Vyatka Rye [in Russian] (Leningrad, 1955).

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THE RELATION BETWEEN GLYCOLIC ACID OXIDASE AND POLYPHENOL OXIDASE

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At the present time much is known of the enzymes participating in the respiration of organisms. Many of them have been studied from the point of view of their composition and their mechanism of action. It is also known that organisms often contain simultaneously several enzymes, for example oxidases, whose functions are the same, but whose mechanisms of action are different. The experimental material accumulated on respiratory enzymes makes it possible to investigate the relationships between various enzymes and to study the effect of external factors on these relationships. Facts obtained in such an investigation will provide new insights into the nature of life and the tools for a more intelligent control of nature. In this study we shall present certain experimental material relating to this question. This material was in part obtained by us recently and in part obtained by one of us earlier [1a]. We have studied glycolic acid oxidase and polyphenol oxidase, enzymes which are widely distributed in plants [1b, 2].

METHODS

The activities of glycolic acid oxidase and polyphenol oxidase were determined in the Warburg manometric apparatus; quinone was determined by the ordinary iodometric method [1d] and glyoxalic acid by the colorimetric method [1e]. Tannins were determined colorimetrically with Folin's reagent [3]. This same reagent was used in the determination of hydroquinone, for which a calibration curve was constructed with chemically pure hydroquinone. Caffeic and chlorogenic acids were determined colorimetrically with Hupfner's reagent [4]. Colorimetric measurements were made with an FEK-N-54 colorimeter.

Polyphenols were identified by paper chromatography using an n-butanol–acetic acid–water solution in the ratio 40:10:50. The upper butanol layer was used. The spots were made visible by spraying the paper with a 0.1 N solution of $AgNO_3$ in 10% ammonia. We have previously described identification of caffeic and chlorogenic acids [5].

EXPERIMENTAL PART

It was previously shown by one of us that glycolic acid oxidase in a centrifuged homogenate of barley leaves is inhibited by polyphenol oxidase substrates—pyrocatechin or hydroquinone [1e]. Polyphenol oxidase has not been detected in barley leaves by ordinary methods [1f]. On the other hand, glycolic acid present in barley homogenates stimulates the conversion of p-benzoquinone [1g].

In leaves of a number of plants which contain large amounts of oxidizable substances of the polyphenolic type, glycolic acid oxidase could not be detected by ordinary methods [1c], but in kok-sagyz [1g, h] and tobacco [1b, 6], for example, both glycolic acid oxidase and polyphenol oxidase are found.

In the light of these facts it seemed of interest to study plants containing polyphenol oxidase, and those lacking it, in relation to whether they contain substrates of this enzyme and in relation to the glycolic acid oxidase activity.

TABLE 1

Content of Caffeic + Chlorogenic Acid and of Tannins, and Glycolic Acid Oxidase Activity in Plant Leaves

Plants	Percent of absolute dry wt.		Glycolic acid oxidase activity, μ l O_2 per hr
	tannins	caffeic + chlorogenic acid	
Barley:			
etiolated	0	0.25*	140
green	0	0.29	230
Coriander, seedlings:			
1st leaves–green	1.06	—	70
1st leaves–etiolated	0.40	—	70
2nd leaves–green	1.304	0.69	85
leaves of mature plants	0.906	—	120
Mint:			
field	1.81	0.97	0
greenhouse, light-grown	1.06	1.93	—
greenhouse, shaded	0.36	0.48	70
Kok-sagyz	—	—	300

*The R_f value and the failure to yield a color with iron salts indicates that some unknown substances were determined with Hupfner's reagent instead of caffeic + chlorogenic acid.

Data obtained in such a study are presented in Table 1 (average of two parallel determinations). The glycolic acid oxidase activity was determined in centrifuged homogenates prepared by grinding leaves with M/15 phosphate buffer, pH 7.0, on a 1:10 wt./vol.basis. Each manometer flask contained 4.5 ml of homogenate and 0.5 ml of 0.1M sodium glycolate.

Table 1 shows that the presence of an active glycolic acid oxidase is in no way related to the presence or absence of polyphenol oxidase substrates, caffeic and chlorogenic acids and tannins. There are no derivatives of polyphenols, determined as tannins with Folin's reagent. There is no caffeic or chlorogenic acid, while there is a glycolic acid oxidase.

Of the plants investigated, mint contains the greatest amounts of tannic materials and of caffeic and chlorogenic acid.

The amount of these substances depends on conditions of illumination under which the mint is grown. Under normal illumination, more polyphenol derivatives are accumulated and glycolic acid oxidase is undetectable by ordinary manometric methods. In shaded plants, the polyphenol derivative content is smaller than in light-grown plants and glycolic acid oxidase can be detected. With respect to the content of polyphenol derivatives, especially with respect to caffeic and chlorogenic acid content, coriander resembles mint grown in shade, and glycolic acid oxidase is found in this plant. We have previously shown that in the leaves of mint and coriander there is an active polyphenol oxidase [5]. We did not determine polyphenol derivatives in kok-sagyz leaves, but one of us had previously found that such substances are present and that with grinding of the leaves they are converted into relatively stable quinones. One gram of fresh plant material contains an amount corresponding to 2 ml M/200 hyposulfite [1 h].

From this it follows that glycolic acid oxidase may function in plants containing polyphenol oxidase and its substrates, and only at relatively high levels of polyphenol oxidase substrate is it not possible to detect it. This does not mean, however, that it is not present and that it does not function. It is quite possible that in this case special methods of detection are required. It was earlier shown that glycolic acid oxidase is able to reduce quinones and polyphenols [7]. In the light of this there is reason to suppose that where polyphenol oxidase and its substrates and glycolic acid oxidase and its substrates are both present, they may act together according to

the following scheme: 1) polyphenol + 02 + polyphenol oxidase → quinone + H_2O; 2) quinone + glycolate + glycolic acid oxidase → glyoxalic acid + polyphenol. Polyphenol may again be oxidized to a quinone by polyphenol oxidase.

A number of model experiments with various enzyme preparations were performed to test this hypothesis. Leaves of barley and tobacco served as experimental material. It is known that there is a polyphenol oxidase which will oxidize hydroquinone in tobacco leaves. As indicated above, hydroquinone is obtained by the reduction of p-benzoquinone in the presence of glycolic acid and glycolic acid oxidase.

A purified preparation of polyphenol oxidase was obtained from potato by a well known method [8]. This polyphenol oxidase oxidizes hydroquinone only in the presence of small quantities of pyrocatechin. The components of the oxidative system were dissolved in M/15 phosphate buffer, pH 7.0. Reaction volume was 5.0 ml.

Results of oxidation of hydroquinone by potato polyphenol oxidase are as follows:

Components	O_2 absorbed, μl in 60 min
Polyphenoloxidase 2.5 mg, pyrocatechin 3 mg	120
The same 2.5 mg, hydroquinone 3 mg	40
" " 2.5 mg, pyrocatechin 0.03 mg	10
Polyphenol oxidase 2.5 mg, hydroquinone 1 mg pyrocatechin 0.03 mg	200

The relation between glycolic acid oxidase of barley and polyphenol oxidase of potato is shown by the following data:

Components	O_2 absorbed, μl in 60 min
Glycolic acid oxidase	34
The same + hydroquinone and pyrocatechin	26
" " + polyphenol oxidase	32
Polyphenol oxidase, hydroquinone, pyrocatechin	200
Polyphenol oxidase, hydroquinone, pyrocatechin glycolic acid oxidase	42
Glycolic acid oxidase, glycolate	200
The same + glycolate, hydroquinone, pyrocatechin	61

The complete system consisted of glycolic acid oxidase—a centrifuged homogenate obtained as described previously [4]—potato polyphenol oxidase (2.5 mg), hydroquinone (3 mg), pyrocatechin (0.03 mg) and sodium glycolate (4.9 mg). Total volume, 5.0 ml.

From these data it is clear that extracts of barley leaves not only do not oxidize substrates of polyphenol oxidase, but contain inhibitors of potato polyphenol oxidase. This inhibitor (or inhibitors) was removed by dialysis through cellophane, but the glycolic acid oxidase was inactivated. In barley this enzyme is very unstable. In a centrifuged homogenate it is almost completely inactivated within three hours at room temperature; it is completely inactivated after a three-hour dialysis at pH 7.0 at 0 to + 5°. An active enzyme could be obtained by precipitation with ammonium sulfate, but this preparation was completely inactivated by dialysis. Because of this it is not feasible to use barley preparations for study of the relationship between the two enzymes.

Tobacco leaves turned out to be more suitable for this purpose (Table 2).

The extract from leaves was obtained by grinding them in phosphate buffer, pH 7.0 on a 1.5 wt/vol. basis, passing the slurry through gauze, and centrifuging the homogenate 5-10 minutes at 3000 rpm; leaves were also ground and used without further treatment.

Glycolic acid oxidase was obtained by precipitation of the extract prepared as described above with ammonium sulfate, suspension of the precipitate in phosphate buffer and a two-to three-hour dialysis of the suspension to remove ammonium sulfate [7]. The entire operation was carried out at 3-5°. In experiments of the 1st and 2nd series, the complete system consisted of 4.5 ml extract of homogenate, 3 mg hydroquinone, and

TABLE 2

Relation between Glycolic Acid Oxidase of Tobacco and Polyphenol Oxidase of Potato

Components	O_2 absorbed, μl in 60 min	Hydroquinone destroyed, mg	Quinone produced, mg	Glyoxalic acid produced, mg
Experiment 1				
Extract from leaves				
+ hydroquinone	224	1.60	0.42	0
+ glycolate	85	—	0	0.15
+ hydroquinone + glycolate	224	0.80	0.24	0.31
Experiment 2				
Homogenate from leaves, hydroquinone	180	—	—	—
Dialyzed homogenate, hydroquinone	17	—	—	—
Dialyzed homogenate, pyrocatechin	17	—	—	—
Experiment 3				
Polyphenol oxidase, hydroquinone, pyrocatechin	400	1.70	0.31	0
Glycolic acid oxidase, glycolate	357	—	0	3.8
Polyphenol oxidase, hydroquinone, pyrocatechin, glycolic acid oxidase	90	0.74	0.53	0
Polyphenol oxidase, hydroquinone, pyrocatechin, glycolic acid oxidase, glycolate	380	0.48	0.15	5.0
Polyphenol oxidase, hydroquinone, pyrocatechin, glycolate	390	1.76	0.15	0

4.9 ml sodium glycolate; total volume was 5.0 ml of a glycolic acid oxidase solution, 5 mg potato polyphenol oxidase, 3 mg hydroquinone, 0.03 mg pyrocatechin, and 4.9 mg sodium glycolate; total volume was 5.0 ml.

Table 2 shows that extracts of tobacco leaves contain glycolic acid oxidase and polyphenol oxidase. The latter oxidizes hydroquinone without the addition of a small amounts of pyrocatechin does not reactivate the enzyme. It was also observed that glycolic acid oxidase obtained by precipitation with ammonium sulfate does not contain polyphenol oxidase after dialysis, and therefore potato polyphenol oxidase was used in experiments with it.

As Table 2 shows, when the two oxidative systems are incubated together hydroquinone is oxidized to quinone and glycolic acid to glyoxalic acid, i.e., both systems are active. This, however, still tells nothing of the relationship between the two systems. On the basis of the data it may be supposed that either there is an oxidation of hydroquinone to p-benzoquinone with the participation of molecular oxygen and polyphenol oxidase, and also an oxidation of glycolic acid with the participation of molecular oxygen and glycolic acid oxidase, or glycolic acid is oxidized completely or partially by quinone formed in the oxidation of hydroquinone.

In the latter case all or a significant part of the oxygen is absorbed by the polyphenol oxidase system, and the glycolic acid oxidase does not activate molecular oxygen ora activates it to a smaller extent than does polyphenol oxidase.

It is also evident from Table 2 that the oxygen absorption of the two enzyme systems together is not equal to the sum of their absorptions separately; under these conditions the amount of oxygen absorbed is equal to that absorbed by either system alone. At the same time there is a smaller consumption of hydroquinone and accumulation of benzoquinone than when polyphenol oxidase is acting alone. Glycolic acid (Table 2) does not affect the activity of polyphenol oxidase, but glycolic acid oxidase inhibits it. This is shown by the fact that polyphenol oxidase activity is reduced. On the other hand, as shown on page 608, substrates of polyphonol oxidase inhibit glycolic acid oxidase.

These facts would seen to indicate that when incubated together glycolic acid oxidase and polyphenol oxidase act in parallel and that their relationship consists of a certain reciprocal inhibition. However, as was shown earlier [7], glycolic acid oxidase, in the presence of quinone, oxidized glycolic acid more readily with

quinone than with molecular oxygen. The possibility cannot be ignored, therefore, that in the system under consideration glycolic acid is oxidized by quinone. In this case, the drcreased consumption of hydroquinone may be only apparent since oxidized hydroquinone can be regenerated by reduction of the quinone formed by the glycolic acid–glycolic acid oxidase system.

In this case, oxygen absorption would be dur solely or to a large extent to polyphenol oxidase activity. As to the truth of the matter, we cannot yet give an answer on the basis of existing data. Probably kinetic studies would be of help in the solution of this problem. It is possible that the existence and the rate of a given reaction in this system will depend on the concentration of components.

SUMMARY

Glycolic acid oxidase was found to function in the leaves of plants in which polyphenol oxidase and its substrates could not be detected (barley), as well as in the leaves of plants containing polyphenol oxidase and substrates (tobacco, mint, coriander, kok-sagyz).

Glycolic acid oxidase could not be detected by the manometric method in leaves of plants (mint) containing polyphenol oxidase and relatively large amount of substrates of this enzyme.

Polyphenols and polyphenol oxidase substrates inhibit the glycolic acid oxidase from barley leaves. A dialyzable inhibitor of polyphenol oxidase from potatoes is contained in barley leaves. Thus, with respect to glycolic acid oxidase of barley leaves the polypyenol oxidase system is an antagonist. Moreover, barley leaves possess a means of protecting glycolic acid oxidase from the injurious effect of the polyphenol oxidase oxidative system on it.

The glycolic acid oxidase system and polyphenol oxidase system simultaneously function in tobacco leaves. Here a definite relationship exists between them. The mechanism of this relationship is discussed.

LITERATURE CITED

[1] P. A. Kolesnikov, a) The Biochemistry of Respiration of Green Cells. Doctoral dissertation [in Russian] (A. N. Bakh Inst. of Biochemistry, SSSR); b) Usp. sovrem. biol., 38, 133 (1954); c) Doklady Akad. Nauk SSSR, 112, 5, 909 (1957); d) Doklady Akad. Nauk SSSR, 84, 4, 452 (1952); e) Biokhimiya, 13, 370 (1948); f) Doklady Akad. Nauk SSSR, 71, 6, 1085 (1950); g) Izv. Akad. Nauk SSSR, Ser. Biol. 4, 88 (1950); h) Doklady Akad. Nauk SSSR 85, 4, 240 (1952); i) Doklady Akad. Nauk SSSR 71, 6, 1085 (1950).

[2] C. R. Dawson and W. B. Tarpley, In J. B. Sumner and K. Myrbäck, The Enzymes, N. Y., 2, p. I, 454 (1951).

[3] H. Friedrich, Pharmazie, 9, No. 3, 240 (1951).

[4] A. R. Guseva and M. G. Borikhina, Biokhimiya, 20, 105 (1955).

[5] P. A. Kolesnikov and S. V. Emenova, Fiziol. Rastenii, 3, 480 (1956).

[6] D. M. Mikhlin and P. A. Kolesnikov, Biokhimiya, 12, 452 (1947).

[7] P. A. Kolesnikov, E. I. Petrochenko, and S. V. Zoré, Doklady Akad. Nauk SSSR, 123, 4 (1958).

[8] F. Kubowitz, Bioch. Leitschr. 299, H. 1-2, 32 (1938).

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THE SET OF CONCEPTS (TERMINOLOGY) RELATING TO PLANT ONTOGENESIS

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The study of plant ontogenesis has moved forward significantly in the last 30 years. In addition to the general theories of I. V. Michurin concerning stages of plant development, and also the theories of ecological-physiological stages of development proposed by T. D. Lysenko, certain other matters have been widely discussed; among these are the question of qualitative changes associated with maturity, designated aging and rejuvenescence by N P. Krenka, the questions of the relationship of organs to each other and the reciprocal influences exerted among them during ontogenesis, etc. A number of new concepts have entered the scientific domain; such are the concepts of aging and rejuvenescence which have different meaning for different workers. Several studies have been published in which one of the most complex problems of plant ontogenesis, the interrelationship of age changes in plants and the progression of ecological-physiological stages has been considered [1-7].

The need for generalizing and synthesizing all the factual data on plant ontogenesis is becoming more and more pressing [4, 5, 7-9].

This makes necessary a revision and a more accurate definition of the concepts associated with plant ontogenesis. Regarding knowledge as an endless approximation of thought to the object, V. I. Lenin wrote: "Human concepts are not immutable, but are constantly changing, merging into one another, and without this they would not express the vital quality of life. The analysis of concepts, their study, the artificial manipulation of them' (Engels) demands at all times the study of their development, their relations, their transitions" [Philosophical Notebooks, Gospolitizdat, (1947), p. 237].

Lenin defined a concept as the "account of individual aspects of movement, of individual drops (= things), of individual 'currents' etc." (p.122), while "truth is built up from all the aspects of a phenomenon, its action, and its relations with other phenomena" (p.169).

Every brancy of science rests first of all on a system of concepts embracing the entire body of factual knowledge in that branch. Scientific concepts are, however, always part of a definite system which comprises a definite hypothesis or theory, and therefore they have a limited area of application. In the extension and alteration of our factual knowledge of a subject, a new set of concepts must be created, for which it is necessary to reformulate, redefine and alter earlier held concepts. This whole position was clearly formulated in respect to the concepts of physics [10], but it is relevant to plant ontogenesis as well.

Concepts are expressed in words comprising the terminology of a given science. It is necessary that scientific terminology be rigidly defined, i.e., that each term correspond to a definite idea, which is the same for everyone; otherwise it would be impossible for workers to understand each other. Terms have therefore been defined. Unfortunately, however, this is often not successful, especially in those cases where words are used scientifically which are also used in ordinary speech.

The plant physiology section of the Second Delegates' Congress of the All-Union Botanical Society, meeting May 9-15, 1957, in a consideration of the problem of individual plant development, agreed on the necessity of making the terminology of plant ontogenesis more accurate. We therefore present for consideration certain views on this problem.

The Concept of Individual Plant Development

The term, "individual plant development" is used in textbooks and in the bulk of botanical and agricultural literature in the sense in which Lysenko used it [11]: "By development of a seed plant we understand the path of obligatory qualitative changes in cellular contents and organ-forming processes which the plant follows from the sowing of the seed to the maturation of new seeds" (p 20). In trying to alter this definition so that it be applicable to plants which do not form seeds, Genkel' and Kudryashov [12] proposed this definition: "By development of a plant is understood that path of obligatory qualitative changes of cellular contents which the plant follows from the reproductive cell (zygote, spore, the organism itself) to the formation of new reproductive cells".

The emphasis on the fact that the essence of development is the qualitative change in cellular contents is correct. However, both definitions bear the stamp of their origin: they were formulated for annual plants in which the formation of new seeds correspond with the end of the life cycle. For perennials they will be incomplete and will obviously embrace only part of the life cycle, namely the reproductive development. These definitions should continue to be used in this sense.

With respect to individual plant development in the wide and complete sense of the term—as the entire process of change in the organism during its life—this has come in recent years to be designated by the term "ontogenesis" in correspondence with its meaning in biology as a whole [13].

Thus, plant ontogenesis, or individual plant development in the wide sense of the term, denotes the complex of regular changes occurring in the organism from its birth to its natural death.

Such an extension of the concept of "individual plant development" is necessary for a more complete study of all the aspects of this development, and not only the reproductive aspect.

During ontogenesis the following phenomena occur: growth and differentiation, organ formation (organogenesis, morphogenesis), qualitative changes associated with maturity (aging and rejuvenescence), the passage of ecological-physiological stages, reciprocal influence of organs, etc.

The formation of plants, especially higher plants, proceeds additively. This means that they are both single entities and composite organisms. Therefore a distinction should be made between the ontogenesis of the plant taken as a whole and the ontogenesis of its organs, which are formed in sequence.

Growth and Organ Formation

Growth is an irreversible increase in the size which is based on the formation of cells, intercellular materials, and new organs. As a rule it is accompanied by an increase in dry weight, as T. D. Lysenko pointed out [11]. It is impossible to overlook the observation of Sabinin [14] that growth is qualitatively diverse, since it is indivisibly linked with differentiation and organogenesis.

Differentiation is the increase in the complexity of the organism, the specialization of its cells, tissues and organs which arise during ontogenesis.

A. A. Sapegin proposed two stages in organ formation: the preparative stage (stage of determination, i.e., the creation of internal conditions required for formation of the organ) and the completion stage (morphologically expressed). The author is in agreement with Lyubinskii's remarks [4] on the profound significance of these concepts and the processes corresponding to them.

D. A. Sabinin [14] first suggested that the vernalization stage plus the light stage should be regarded as a time of ecological-physiological determination of reproductive organs in plants. He proposed the introduction of the concept of organ determination, which had long been used in animal development studies, into plant physiology. We believe this proposal should be adopted, the determination of organs in plants being understood as the sum total of effects of genetic, correlative, age and ecological-physiological factors which unite to bring about organ formation.

The determination of reproductive organs, i.e., organs of sexual and vegetative reproduction, is of especial significance in plant ontogenesis.

Age Changes in Plants

As Krenke pointed out [15,16], calendar age refers to time elapsed from birth or formation of a new individual, and physiological age refers to a definite physiological condition of the plant as a whole or of its parts, this being determined not only by calendar age but also by a number of external and internal factors.

At present, the term "physiological age" is used in two senses in scientific writing: 1) As a synonym for "ontogenetic age", i.e., in the widest sense, including the entire complex of qualitative changes occurring in plant ontogenesis. This concept embraces both the progressive changes associated with ecological-physiological stages in Lynsenko's sense and the changes associated with physiological aging as such (according to Krenke). 2) In the narrow sense—as a term denoting the condition arising as a result of individual qualitative changes in individual organs and in the plant as a whole together with progressive ecological-physiological changes, the two types of change being interrelated [1].

The ambiguity of the terms "physiological age" and "physiological aging" leads to great confusion. In order to prevent this, we propose the use of these terms only in the narrow sense, and for the entire complex of qualitative changes occurring in ohtogenesis we propose the terms "ontogenetic age" and "ontogenetic aging".

On the other hand, the widespread use of the term "progressive" as a synonym for "ontogenetic" has also caused confusion. Lyubinskii's book [4] is a striking example of the difficultues arising with extensive use of this term.

In agreement with the repeated assertions of N. A. Maksimov, the term "progressive changes" should be used only in the sense of Lysenko; for greater definiteness, therefore, it is appropriate to use the expression "progressive ecological —physiological changes".

Thus, by qualitative ontogenetic changes should be meant physiological and progressive ecological—physiological changes.

Physiological changes are manifested in the form of aging or rejuvenescence, which are dialectically opposed processes.

Senescence — This is a gradually developing condition of an organism, its organs, tissues and cells, which is expressed as a reduction of metabolic level and change in metabolic character, a decline in rate of protein synthesis and other anabolic processes as well as growth processes and physiological activity, an accumulation of little-active structures, and a decrease in resistance to various injurious factors. Senescence is a stage in ontogenesis of the organism as a whole and also of individual organs,tissues and cells.

The process of development of this condition is called aging. In the organism as a whole, this is usually observed at the beginning of the senescent period. Aging and death of individual cells (and in plants, even in individual metameric organs) take place, however, from the earliest stages of ontogenesis, i.e., they are constantly going on throughout life.

Rejuvenescence of plants —This is the neoformation of more juvenile structures (organs), and also the heightening of metabolic activity in the form of protein synthesis and other anabolic processes, growth processes and physiological activity. During reproduction by sexual or vegetative means, and also during regeneration from callus there is a profound rejuvenescence, with return to a developmental level which is prior to all ontogenetic changes, including ecological-physiological changes. In other cases the degree of rejuvenescence may be different [17].

The progressive change in the ontogenetic condition of an organism, beginning from its birth, cannot be called "aging", as in Krenke's writings. The term "progressive aging" (Kazaryan [3], Lyubinskii [4]) is also unsuitable, since aging denotes a process of weakening and physiological decline and progressive changes refer to specific ecological-physiological changes in the sense proposed by Lysenko. It is appropriate to use the term "progressive development" or "passage through a given stage".

When an organism is exposed to unfavorable external conditions (water deficiency, high or low temperature, disruption of nutrition), there also appear temporary (reversible) or permanent (irreversible) qualitative changes which are somewhat analogous,physiologically, to those occurring during ontogenetic aging. The use of the term "induced physiological aging" in these cases is unfortunate, since "normal ontogenetic aging" may also be hastened or retarded by external conditions, although it is for the most part controlled by internal factors.

Division of Ontogenesis into Periods

It is possible to distinguish qualitatively different stages in the ontogenesis of higher plants from different points of reference: overall (age), ecological-physiological, morphogenetic, and organogenetic.

The chief stages of general biological significance in the ontogenesis of higher plants are those proposed by Michurin: embryonic, juvenile, reproductive and senescent; these are most clearly shown in perennials. In annuals and biennials of the moderate zone, the ecological physiological stages of Lysenko((vernalization and light) are clearly manifested. Morphological stages, determined by morphological criteria, embrace the entire ontogenetic sequence, which occurs in annuals and in the yearly developmental cycle of perennials. In considering reproductive development it is also expedient to distinguish stages of organ formation (according to Sapegin, Zabluda and Kuperman).

All these approaches to the ontogenesis of higher plants, a many-faceted process, are based on solid theoretical foundations and are fully suitable for practical application since they open the way for a more extensive control of plant life in human interests.

Scientific progress in the knowledge of plant ontogenesis will depend chiefly on further investigations of ontogenesic patterns. A correct generalization of the body of data thus far obtained is of substantial value, however. One of the aspects of this generalization is a systematization of the concepts and terminology relating to plant ontogenesis. It is possible that our suggestions may not be entirely valid for a given situation and may require considerable revision or extension. One thing is true: this matter deserves attention. We are far from thinking that we have introduced a settled system of concepts which will meet the needs of every scientist: in the development of the subject of ontogenesis there will exist different shades of usage of concepts and terms by different workers. However, it is entirely possible and also vitally necessary to come to an agreement on terminology at the earliest time.

LITERATURE CITED

[1] P. I. Gupalo, Byull. Mosk Obshch. Ispyt. Prirody, Otd. biol. 62(5) (1957).

[2] L. G. Dobrunov, Physiological Changes in Plant Ontogenesis [in Russian] (Alma-Ata, 1956).

[3] V. O. Kazaryan, Development by Stages and Aging in Annuals [in Russian] (Erevan, 1952).

[4] N. A. Lyubinskii, Physiological Bases of Plant Vegetative Reproduction [in Russian] (Kiev, 1957).

[5] G. Kh. Molotkovskii, "Reciprocal inhibitions of plant organs as a basis of plant development." Chernovitskii Gos. Uch. zap. biol. fak-ta Chernovitskogo gos. Univ 1, (1948).

[6] V. V. Skripchinskii, Byull. Moskov. Obshch. Ispyt. Prirody, Otd. Biol. 61 (4 and 5) (1956).

[7] M. Kh. Chailakhyan, Basic Patterns of Ontogenesis in Higher Plants [in Russian] (Izd. AN SSSR, 1958).

[8] P. I. Gupalo, Usp. Sovrem. Biol. 38, 111 (1954).

[9] M. Kh. Chailakhyan, Thesis of the Proceedings of the Plant Physiology Section of the Delegates' Congress of the All-Union Botanical Society, May 9-15, 1957 [in Russian.

[10] T. A. Brodi, Voprosy filosofii 2, 83 (1957).

[11] T. D. Lysenko, Agrobiology [in Russian] (Sel'khozgiz, 1948).

[12] P. A. Genkel' and A. V. Kudryashov, Botany [in Russian] (Uchpedgiz, 1952).

[13] G. K. Khrushchov and P. A. Baranov, "Ontogenesis" [in Russian] Large Soviet Encyclopedia, 31 (1955).

[14] D. A. Sabinin, Plant Mineral Nutrition [in Russian] (Izd. AN SSSR, 1940).

[15] N. P. Krenke, The Theory of Cyclical Aging and Rejuvenescence in Plants and Its Practical Application [in Russian] (Sel'khozgiz, 1940).

[16] N. P. Krenke, Plant Regeneration [in Russian] (1950).

[17] N. I. Dubrovitskaya and A. N. Krenke, Usp. Sovrem. Biol. 36, 64 (1953).

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BRIEF COMMUNICATIONS

MOBILIZATION AND TRANSLOCATION OF ORGANIC SUBSTANCES IN WHEAT SEEDLINGS AFTER X-RAY IRRADIATION

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It has been previously shown that the growth of winter wheat 599 seedlings is completely suppressed by irradiation at 3000 r, while other physiological processes are undisturbed and the seedlings remain turgid and green and accumulate sugar and other photosynthetic products; even at very large doses, 1,000,000 r, the plants remain alive [1,2]. The death of plants may result from irradiation [3, 4, 5].

This paper presents data on the mobilization and translocation of organic substances in wheat seedlings which have been irradiated with growth-inhibiting doses.

Winter wheat 599 seedlings grown in the dark from seed which had been irradiated with doses known to suppress growth, 50,000 and 100,000 r, were used. That growth had been completely suppressed could be assumed from the disappearance of meristems in the root tips and leaf bases; this was also externally evident in the thick mat of root hairs over the entire surface of the root down to the root cap, a situation which is never found in roots with a terminal meristmatic zone, and in the absence of any elongation of leaves removed at the level of the coleoptile after the meristematic cells had undergone vacuolization and extension [6].

Seeds which had been selected for uniform size were irradiated in an RUM-3 apparatus without a filter at a focal distance of 20 cm and a dosage rate of 270 r/minute. Seeds were then laid out in Petri dishes, 100 to a dish, in 12 replicate groups. The same procedure was followed with unirradiated seeds. Ten ml of tap water was added to each dish and the dishes were placed in a dark incubator at 25 + 2°. The seedlings were washed daily in order to prevent the development of mold and enough fresh water was added so that only the roots were immersed. They were kept under these conditions 10 days. They were then placed in an oven at 80-90° for 15 minutes to inactivate them, the grain was removed, and the seedlings were dried to constant weight at 105°.

Results of the experiment are as follows:

Dry wt. of 100 seedlings, in g	Difference in wt. between seedlings and embryos of imbibed seeds
Original wt. of 100 embryos after an 18-hr soaking of seeds 0.103 ± 0.103	–
Two-day-old seedlings from unirradiated seeds 0.141 ± 0.005	0.038 ± 0.014
Ten-day-old seedlings from unirradiated seeds 1.233 ± 0.012	1.130 ± 0.018
Ten-day-old seedlings from seeds irradiated at 50.000 r 0.588 ± 0.021	0.485 ± 0.025
Ten-day-old seedlings from seeds irradiated at 100.000 r 0.341 ± 0.071	0.238 ± 0,072

The increase in dry weight of seedlings during the period in the dark is based on the weight of embroys from soaked seeds. The weight of two-day-old and ten-day-old seedlings gives some idea of the dynamics of accumulation of dry matter in unirradiated seedlings.

During the time the seedlings were kept in the dark their dry weight increased. Two-day-old seedlings from unirradiated seeds weighed approximately 40% more than the embryos from soaked seeds, and ten-day-old seedlings had increased their weight almost 12-fold. With irradiation, the accumulation of dry matter was reduced. But this process was still not completely inhibited. The flow of organic materials from the grain continued and the seedlings increased in dry weight. The rate at which this took place was, however, decreased with an increase in radiation dose.

The increase in dry weight in the absence of growth may be explained thus: Organic materials passed from the endosperm into all the meristemic cells of the embryo, and not being utilized in growth, were stored by the cells, which accounts for their vacuolization [6]. In this way the dry weight of each cell was increased. The weight of the seedling as a whole was also increased. This process was discussed by us earlier [6], and we will not consider it further.

It is important to consider the following. The increase in seedling weight was due to the flow of organic materials from the endosperm, where they were stored as insoluble substances such as starch, proteins, and fats. Their conversion into soluble form, in which they were translocated to the embryo, clearly indicates that this process is not inhibited even by irradiation with the large doses we used. However, the rate of this process is dependent on dose, and is less for larger doses.

The largest dose employed was 100,000 r. This dose did not completely suppress the mobilization and translocation of organic materials from the endosperm to the embryo. Therefore it was not yet terminal for these processes.

It has been established that irradiation of seeds of winter wheat 599 by doses of 50,000 and 100,000 r, which completely abolish growth, did not suppress the mobilization and translocation of organic materials from the endosperm. With an increase in dose this process was retarded.

LITERATURE CITED

[1] I. M. Vasil'ev and N. D. Rybalka. "The effect of x-ray irradiation on photosynthesis in wheat plants." Doklady Akad. Nauk SSSR 121, 1 (1958).*

[2] I. M. Vasil'ev, N. D. Rybalka and Ts'in Su-Yün. Doklady Akad. Nauk SSSR 119, 1 (1958). *

[3] G. W. Collins and L. R. Maxwell, Science 83, 2155, 375 (1936).

[4] A. S. Sussman, J. Cellular and Compar. physiol. 42, 2 (1955).

[5] M. Lefort and L. Ehrenberg, Arkiv bot. 3, 1-2, 121 (1958).

[6] I. M. Vasil'ev and Ts'in Su-Yün, Biofizika, 3, 4 (1958).

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* See English translation.

A METHOD OF INVESTIGATION OF THE ROOT SYSTEM OF CORN UNDER FIELD CONDITIONS

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The elucidation of the roles of seed roots, secondary roots and prop roots in the growth and development of corn is of great interest to both physiologists and plant growers. A schematic diagram of the structure of the root system of corn is shown in Fig. 1.

We have developed a method which enables us to study the role of the seed roots and secondary roots in corn under field conditions.

We succeeded in growing plants on seed roots alone by the following method. A soaked corn grain was planted in moist soil at a depth of 6-7 cm. To prevent the seed from drying out we covered the ground with a thin layer of humus.

Two or three days after the appearance of the first two leaves (seed and secondary root formed) the soil around the roots was carefully loosened and removed to a depth 1-2 cm below the base of the coleoptile. As is well known, there is between the seed and the base of the coleoptile a special internode, the mesocotyl. It would be more correct to call it the epicotyl since it is located above the single cotyledon—the scutellum.

To prevent the penetration into the soil of the coleoptilar and secondary roots, which are formed above the mesocotyl, a disk of zinc-plated tin 12 cm in diameter with an aperture in the center was fixed in such a position that the mesocotyl passed through the central opening. This is shown in Fig. 2. The disk was first cut into two equal halves. An incision 3 mm wide and about 2 cm long was made from the center toward the periphery. In placing the disk halves in position, first one was slipped onto the mesocotyl at the place of incision and then the other was slipped on from the opposite side. As a result, the mesocotyl was made to pass through the center of the disk. Below the disk were the lower part of the mesocotyl, the seed and the seed roots. The disk was then carefully covered with soil. The base of the stem was shaded to prevent heating of the soil by the sun. Periodically the soil was removed from around the stem base and the secondary roots which had formed above the disk were carefully excised.

The tendency of corn plants to fall down presents great difficulties in growing them on seed roots alone. It was therefore necessary to provide them with support in order to keep them in an upright position. Even then, especially in strong winds, the mesocotyl was often broken and the plant died. This should be kept in mind when using this method.

For observations of growth and development in corn growing on secondary roots only, a method was developed for removing the seed roots. In this case we took into consideration that the very early growth of young plants, before the formation and functioning of secondary roots, depends on the seed roots exclusively. It is therefore impossible to remove them at early stages. We also took into consideration that the time at which the seed roots are removed and the plant becomes dependent on the secondary roots is of great importance for the plant's future development.

In this connection, it is very important for experimental purposes to isolate the seed roots from the rest of the root system at the appropriate time. Let us recall that between the seed roots and the remaining portion of

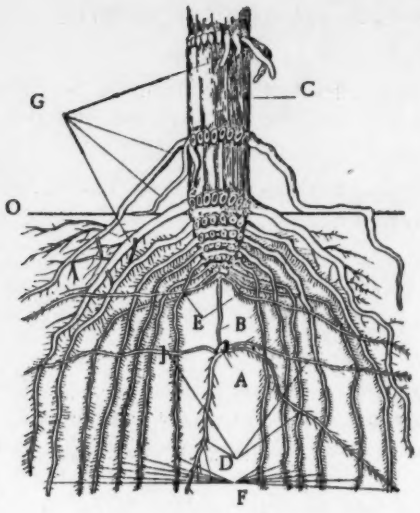

Fig. 1. Structure of the root system of Odessa 10 corn. O—Soil surface; A—seed; B—mesocotyl; C—aerial portion of the stem; D—seed roots; E—coleoptilar roots; F—secondary roots; G—prop roots.

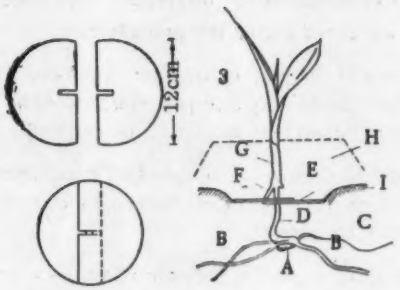

Fig. 2. Experimental design for growing corn plants on their seed roots alone. 1) the two disk halves with the central incision; 2) the two halves in contact so as to produce a central opening for the mesocotyl to pass through; 3) position of the disk in the experiment; A—seed; B—radicle; C—branch seed roots; D—mesocotyl; E—disk; F—incipient secondary roots; G—coleoptyle, H—shading for the plant base; I—soil surface.

the root system of corn is the mesocotyl (see Fig. 1). Severance of the mesocotyl at the proper time thus prevents the seed roots from supplying the plant with water and nutrients.

The mesocotyl was severed with a metallic strip of tin in the form of an elongated trapezoid about 20 cm in length (Fig. 3) with an oblique incision in the lower portion.

Upon appearance of the shoots the soil was removed down to the middle portion of the mesocotyl. The metal strip was slipped onto the mesocotyl and placed in an inclined position so as to reach the soil surface. The plant and the strip were then covered with soil, with the upper end of the strip left protruding above the surface. At the desired time the strip was pulled up by its free end. In this way the mesocotyl was severed and the seed roots isolated.

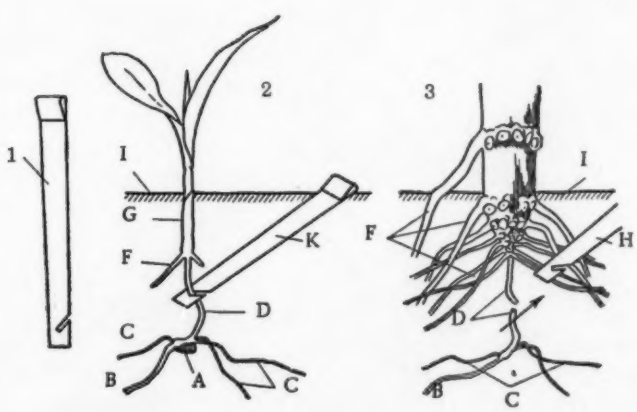

Fig. 3. Method of severing the mesocotyl. 1) Metal strip; 2) drawing to show position of strip; 3) drawing of plant after isolation of the seed roots by severance of the mesocotyl. Letters denote the same things as in Fig. 2. K—metal strip. Age of the plant—12 leaves.

In our experiments we isolated the seed roots at the 3-, 4-, 6-, 8-, and 12- leaf stages. No injury to the other roots was noted during this procedure.

The role of the prop roots, which are formed at the lower stem nodes, is still not clear. Do they function only as support, or do they also participate to some extent in supplying the plant with water and mineral nutrients; it is of great importance to clarify this problem.

In order to do this, we designed an experiment with the following treatments: 1) control (untreated); 2) systematic removal of prop roots without supporting the stem; 3) systematic removal of the prop roots with provision for artificial support.

Plants from which the prop roots had been removed and to which no support had been given could not remain erect. Therefore the study of the role of the prop roots in water and mineral uptake must be made only when support is provided.

When cultivators pass down the rows in corn fields the roots are often injured. The extent of injury depends, first, on the distance between the blades of the cultivator and the plant, and second, on the depth of cultivation. In view of this, a special "protective zone", i.e., a zone between the plant and the blades of the cultivator, is set up. For corn, this zone is usually 10-12 cm in each direction. In most cases, the first cultivation is to a depth of 10 cm, and the succeeding cultivations to a depth of 5-6 cm. Direct observation of the extent of injury to the root system after cultivation is very complicated. Therefore we used the following method of studying the effect of root injury.

The roots were cut with a long sharp knife or a straight pointed trowel on four sides of the plant (as the sides of a square). Distances between plants were 5, 10 and 15 cm, and the roots were cut at 5 and 10 cm. The roots may be cut at various stages in plant development, at various intervals, at various depths, etc., to simulate the cultivation regimes which are employed in different fields.

Under differing soil-climatic conditions, the results of similar investigations will of course be different. The analysis of such results will provide a theoretical foundation for the determination of the necessary size of the protective zone, the suitable depth and frequency of cultivation, etc. The theoretically established procedures must be subsequently tested in the field with the equipment commonly used in plant cultivation.

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PENETRATION INTO PLANTS OF HERBICIDES AND THEIR INFLUENCE ON PHOSPHORUS UPTAKE

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The effect of chemical preparations used as growth stimulators and herbicides depends to a large extent on the rate of penetration into plants and the distribution within the plant. It is therefore natural that an investigation of these processes should yield information on the mechanism of stimulating, inhibiting and herbicidal actions of these preparations.

There are still only a few experimental investigations of these problems. It was shown that 2,4-D which is labeled with C^{14} in the carboxyl group is translocated mainly from the treated leaves downward through the stem [1, 2]. The same conclusion was made by other workers [3], who noted that 2, 4-dichloro-5-iodophenoxyacetic acid containing radioactive I^{131} is translocated from the treated leaves of bean seedlings downward through the stem and is accumulated in the hypocotyl. Other experiments [4] have shown that the translocation of 4-iodophenoxyacetic acid labeled with I^{131} in tomato plants is dependent on the metabolic activity of the tissues. It was shown that this compound is concentrated to the greatest extent in meristematic tissues, which have a high rate of metabolism, and to the least extent in mature tissues, which have a low rate of metabolism; it is accumulated to a significantly smaller degree in the growing points of plants whose metabolism has been depressed by sprinkling with a solution of the diethanolamine salt of maleic hydrazide. It has been shown that herbicides penetrate different plants at different rates [5] and that a surface active agent increases the penetration [6].

It has been experimentally demonstrated [7, 8] that in essence the effect of herbicides is to disrupt the uptake of mineral nutrients by plants.

In this paper we shall present the results of our studies of the penetration of 4-iodophenoxyacetic acid (4-IPA) into imbibing seeds and growing plants, of the distribution of this compound and the plant material and, finally, of its effect on phosphate uptake.

Experimental material consisted of pea, corn and wheat seeds and also growing oat and sunflower plants. These were exposed to 4-IPA containing I^{131}. The seeds were soaked in a 0.05% solution (activity 720 counts/minute$\cdot$ml solution) for 15.5 and 24 hours; they were then washed in running tap water for five minutes and dried in air, after which they were prepared for study—the seed coat was removed and the embryo dissected out. A 0.2% solution of 4-IPA (activity 540 counts/minute$\cdot$ml solution) was used in the experiments with growing plants. Only the upper leaves were sprinkled, the lower parts of the plants being carefully shielded with cellophane. At definite times the various parts of the treated seeds (20 seeds, replicated three times) and plants (two plants, replicated twice) were taken for analysis. The plant material was prepared for counting according to the method described by Rakitin and Krylov [4].

Data are also presented on the effect of the sodium salt of 2, 4-D and of the isopropyl ester of N-3-chlorophenyl carbamic acid (ICPC) on the uptake of phosphorus from a nutrient solution. Experiments were performed with 8- and 30-day-old oat, sunflower and bean plants. The plants were grown on Knopp's nutrient solution. Some plants were sprinkled with a 0.1% aqueous solution of 2,4-D and others with a 1% or a 0.25% aqueous emulsion of ICPC, using OP-7 as an emulsifier. Following this KH_2PO_4 labeled with P^{32} was added to the nutrient solution. Samples were taken from the plants 24 hours and 5 days later for measurement of radioactivity. The

TABLE 1

Distribution of Radioactive 4-Iodophenoxyacetic Acid (0.05%) in Seeds of Various Plants (amt. of 4-IPA expressed as counts/min • 20 mg dry wt.)

Experimental conditions	Parts of seeds and seedlings	Pea	Corn	Wheat
	First experiment			
Seeds in solution 15.5 hr; count made 4-6 hr after treatment	Seed coat	12	13	-
	Endosperm or cotyledons	34	9	-
	Embryo	80	14	-
	Second experiment			
Seeds in solution 24 hr; count made 5-6 hr after treatment	Seed coat	28	21	27
	Endosperm or cotyledons	99	11	8
	Embryo	125	22	52
Count made 13 days after treatment	Seed coat	46	10	13
	Endosperm or cotyledons	24	11	7
	Embryo	26	20	18

leaves (three from each plant, replicated three times) were first dried and then ground in mortars; from the ground material tablets were prepared according to a method described earlier [4]. At the thickness used it was not necessary to take account of self absorption.

All results were corrected for radioactive decay and for background.

Table 1 shows (second experiment) that after a 24 hour exposure to 4-IPA, peas had absorbed five times more per unit weight than had corn grains. The 4-IPA was unequally distributed among various parts of the seeds: seed coat, endosperm or cotyledons, and embryo. It occurred in the highest concentration in the embryo. The extent of absorption by different parts of the seed varied with the type of plant. Pea embryos absorbed almost six times as much as corn embryos and pea cotyledons seven times as much as corn endosperm, while the seed coats were found to contain approximately the same amount of 4-IPA.

TABLE 2

Distribution of Radioactive 4-Iodophenoxyacetic Acid (0.2%) in Oat and Sunflower Plants (determinations were made two days after treatment. The amount of 4-IPA is expressed as counts/min·20 mg dry wt.)

Plant	Age, in days	Part of plant	Counts/min
Sunflower	30	Middle part of stem with leaves	7
		Lower part of stem	5
		Roots	6
Oat	30	Middle part of plant	6
		Lower part of plant	8
		Roots	5
	14	Middle part of plant	7
		Lower part of plant	8
		Roots	6

TABLE 3

The Effect of Herbicides on the Uptake of Radioactive Phosphorus (P^{32}) in Oat and Bean Leaves (experiment was carried out with 30-day-old plants. The amount of P^{32} is expressed as counts/min·20 mg dry wt.)

Leaves analyzed	Treatments and solution concentrations, %	Oats		Kidney bean	
		counts per min	in % of control	counts per min	in % of control
	On the Second Day after Treatment				
Upper	control	69	100	273	100
	0.1% 2,4-D	39	56	62	22
	1% OP-7	99	143	97	36
	0.25% ICPC + 1% OP-7	92	133	128	46
	On the Fifth Day after treatment				
The same	control	221	100	360	100
	0.1% 2,4 D	142	64	535	148
	1% OP-7	32	14	167	46
	0.25% ICPC + 1% OP-7	104	47	575	159
Lower	control	144	100	102	100
	0.1% 2,4 D	364	252	150	138
	1% OP-7	87	60	93	86
	0.25% ICPC + 1% OP-7	310	215	189	186

It is also apparent from Table 1 that during germination of seeds soaked in a 4-IPA solution the greatest amounts of this compound are taken up by the rootlets, especially in cereal grains (corn and wheat). This circumstance is obviously associated with the fact that the root grows faster than other parts of the seedling during germination.

In the experiment with growing oat and sunflower plants (only the upper plant parts exposed to 4-IPA), the herbicide was translocated down the stem and reached the roots (Table 2).

It was experimentally shown that 2, 4-D, ICPC and OP-7 substantially disrupt the uptake of phosphorus by the plant.

As Table 3 shows, 2,4-D sharply reduces the content of phosphorus in the young leaves of oat and bean, especially the latter. ICPC has a different effect: after exposure to this compound the phosphorus content of bean leaves is also reduced, but in oats it is markedly increased. In time the phosphorus content of young bean leaves of plants which have been treated with 2,4-D increases, while the phosphorus content of young leaves of oat plants treated with ICPC decreases. In growing bean and oat leaves the phosphorus content rises more sharply than in the same leaves from control plants (apparently because of a flow of phosphorus from the young leaves). It should be pointed out that OP-7 depresses phosphorus uptake in both oats and beans.

LITERATURE CITED

[1] R. W. Holley, F. R. Boyle and D. B. Haud, Arch. Biochem. and Biophys. 27 (1950).

[2] R. W. Holley, Arch. Biochem. and Biophys. 35 (1952).

[3] R. J. Linder, J. N. Mitchell and Wood, Science 8, 2889 (1950).

[4] Yu. V. Rakitin and A. V. Krylov, Fiziol. Rastenii 1, 173 (1954).

[5] R. E. Baldwin, V. H. Freed and S. C. Fang, J. Agric. and Food Chem. 2 (1954).

[6] E. W. Hauser, Agron. J. 47, 32 (1955).

[7] M. Ya. Berezovskii and V. F. Kurochkina, TSKhA 22, 380 (1956).

[8] M. Ya. Berezovskii and V. F. Kurochkina, Doklady Akad. Nauk SSSR 113, 2, 458 (1957).*

* See English translation.

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DIURNAL COURSE OF PHOTOSYNTHESIS UNDER ARTIFICIAL ILLUMINATION

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Photosynthesis is related to internal and external factors in a complex way which has not as yet been thoroughly studied. By the former is meant the physiological status of the plant at which all of its functions, including photosynthesis, take place.

Harder [1] points out that photosynthesis may be altered under the influence of internal factors with external conditions being held constant.

The experiments of Danilov [2] and Brilliant [3, 4] have shown that photosynthesis is in the strongest degree dependent on growing conditions. According to the data obtained by Voskresenskaya [5] photophilic plants grown at low light intensity utilize blue light in photosynthesis better than plants of the same species grown in full daylight. Moshkov [6] was able to elicit in mimosa and kidney bean an internal metabolic rhythm in response to an external photoperiodic rhythm. This internal rhythm corresponded precisely with the light rhythm, being expressed as an opening and closing of the leaves, and was persistent for a certain length of time in plants which had been subjected to a different light regime.

Filzer [7] has obtained interesting data. He excised leaves of Polygonum cuspidatum which had been growing under natural conditions and determined their photosynthetic activity under strictly controlled laboratory conditions. It turned out that these measurements reflected quite faithfully the diurnal rhythm of photosynthesis, i.e., they expressed not the direct influence of external conditions of measurement but the physiological rhythm which had been established in the plants. Karmanov and Pumpyanskaya [8] observed a clearly expressed diurnal rhythm of transpiration in cotton and kidney bean plants kept under constant external conditions: temperature, humidity, and light intensity. This rhythm corresponded to natural climatic rhythms and occurred even when the normal course of meteorological events was disturbed, as when the light period was extended.

Out experiments were concerned with the diurnal course of photosynthesis in Perilla ocymoides kept at constant light intensity and at various day lengths.

Novinka oil perilla plants were grown for half a month under constant illumination by direct current fluorescent lamps. Under these conditions they developed a luxurious vegetative growth but failed to flower because of the unfavorable length of day. Plants of the same degree of development were selected for the experiment and were treated in the following manner. Each plant was decapitated and then all leaves and all growing points were removed with the exception of one leaf (sixth-seventh node) and two embryonic axillary shoots (fourth node).

The plants were then placed under light from heat lamps furnished with a water filter. The light intensity at the leaf surfaces was 140-200 watts/m^2, or 11,300-17,800 lux. Four treatments were given: a 12-hour day (from 5 a. m. to 5 p. m.); a 14-hour day (from 5 a. m. to 7 p. m.); a 16-hour day (from 5 a. m. to 9 p. m.); an uninterrupted light period.

After a week, when the plants had become sufficiently adapted to the new conditions, determinations of the diurnal course of photosynthesis were begun. For this purpose chambers of transparent cellulose acetate film were prepared. These were conical in form and were carefully sealed at the apex by the insertion of a rubber

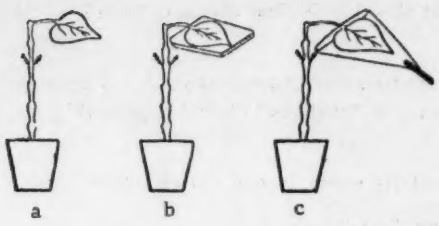

Fig. 1. Preparation of plants for determination of diurnal course of photosynthesis. a—Plant prepared for the experiment; b—leaf enclosed in envelope of cellulose acetate film; c—leaf enclosed in chamber for determination of photosynthesis.

tube (Fig. 1). To further insure a hermetic sealing, the seams, which had been made by heating, and the point of contact of the chamber with the rubber tube were glued together with all-purpose BF-2 glue. At the end of the exposure period the chambers were removed and the leaf was enclosed in an envelope (10× × 10 × 1 cm) of the same cellulose acetate (Fig. 1b) which was supported by a wire running into the soil. In this way the leaves were kept under constant conditions during exposure periods and the intervening periods.

Determinations of photosynthesis were carried out according to the method developed by V. A. Chesnokov and E. N. Bazyrinaya.

Since the leaf's activity is determined by its temperature, and not the temperature of the surrounding air, we measured leaf temperature with a thermocouple.

The values obtained for photosynthetic activity are not high. They reach 4 mg CO^2/ in^2 · hour, although as our previous study on light curves for photosynthesis in perilla had shown, the light intensity which was given was almost twice the saturating intensity. Chesnokov and Stepanova [9] also obtained low values for photosynthesis in tomatoes and cucumbers grown under artificial light.

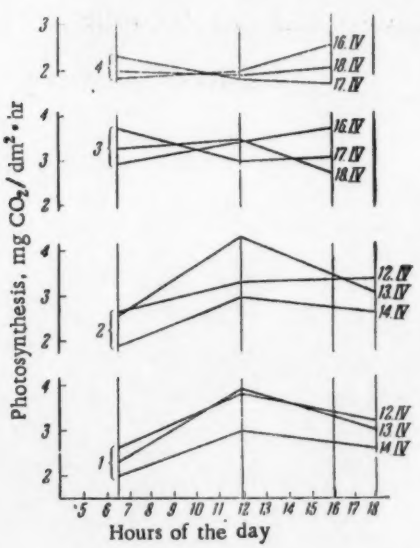

Fig. 2. Diurnal course of photosynthesis in oil perilla. 1) 16-hour day; 2) 14-hour day; 3) 12-hour day; 4) uninterrupted illumination.

An analysis of the numerical data reveals extremely interesting facts. In spite of the constancy of the light and temperature conditions employed, the photosynthetic activity of a leaf did not remain at one level. It exhibited significant fluctuations, these being regular in character in some cases, while in other cases no pattern was discernible (Fig. 2).

The plants fall into groups according to their responses. Those in the first group (treatment 2 and 3, corresponding to curves 2 and 1 in Fig. 2) received a day length close to that characteristic of the regions in which this species is indigenous (in the Far East, at 45° north latitude). Plants of the second group (treatments 1 and 4, corresponding to curves 3 and 4 in Fig. 2) were kept at a day length to which this species is not exposed in nature. Actually, oil perilla is never exposed to a 12-hour day or to constant illumination in nature.

If we examine the course of photosynthesis on 14-to 16-hour days, we see that it is extremely reminiscent of the pattern observed in nature, which is associated with changes in climatic factors: light intensity, and temperature of the surrounding air. Under the laboratory conditions employed, the light intensity was kept strictly constant and the leaf temperature did not vary by more than 1-2° during the period of illumination. Even so, photosynthetic activity in the middle of the day was almost 1.5 times that in the morning; activity in the evening was regularly lower than during the day (Fig. 2, graphs 1 and 2).

We believe that such a reproduction of the diurnal course of photosynthesis in conditions which should not in themselves elicit this pattern of activity is an expression of an internal rhythm. This rhythm is fixed in the

course of evolution under the influence of climatic factors and is transmitted from generation to generation, being reinforced by heredity. At a day length similar to that at which the leaf normally functions, the internal rhythm becomes manifest.

At a day length not usually experienced by the given species, the rhythmic character of photosynthesis is lost and the fluctuations observed are without a regular pattern. A "confused" rhythm appears (Fig. 2, graphs 3 and 4).

A continuous automatic recording of photosynthetic activity would deepen and extend our observations.

In conclusion I wish to thank my adviser, B. S. Moshkov, for his help.

LITERATURE CITED

[1] R. Harder, Planta 11, 263 (1930).

[2] A. N. Danilov, Éksptl. Bot., Ser. IV, 4 (1940).

[3] V. A. Brilliant, Sovet. Bot. 4, 28 (1940).

[4] V. A. Brilliant, Éksptl. Bot., Ser. IV, 8 (1951).

[5] N. P. Voskresenskaya, Tr. Inst. Fiziol. Rastenii Akad. Nauk SSSR 10 (1955).

[6] B. S. Moshkov, Agrobiologiya 4, 125 (1953).

[7] P. Filzer, Jahrb. wiss. Bot. 86, 228 (1938).

[8] V. G. Karmanov and S. L. Pumpyanskaya, Agrobiologiya 6, 117 (1956).

[9] V. A. Chesnokov and A. M. Stepanova, Tr. Inst. Fiziol. Rastenii Akad. Nauk SSSR, 10 (1955).

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CALCULATION OF THE ASSIMILATION AREA OF CUCUMBER PLANTS

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In scientific research it is often necessary to determine the total leaf area of plants, since the leaf is the most important organ of photosynthesis, gas exchange and transpiration. Methods previously employed require the expenditure of a large amount of time, usually involve the removal of the leaves, and moreover, are not absolutely accurate. The most common method of measurement involves outlining the leaf on paper and determining the area by weighing or by other procedures which require special equipment.

Working in hothouses with Klinskii cucumbers (subspecies Chinese, varieta group Klinskii) we made a large number of area determinations without removing the leaves from the plant. This was done in a number of ways by obtaining leaf contours with light-sensitive and moisture-sensitive (treated with cobalt chloride) paper. In none of the procedures, however, was the time required reduced nor was there complete avoidance of injury to the vegetative and reproductive organs.

We therefore attempted to determine leaf surface area by calculations from certain linear measurements (length and width). It was found that there is a constant relation among certain dimensions of the leaf blade which makes it possible to devise a formula for the surface area.

The leaf surface may be divided into regular geometrical figures, triangles and trapezia.

Leaf blades from a single cucumber plant may be divided into three principal groups according to form (Fig. 1): 1) The width (B) is less than the length (A). This form is characteristic of young leaves having an elongated triangular form; 2) The width is equal to or somewhat greater than (not more than 10%) the length. This is most typical of leaves which make up the major portion of the assimilating surface. Such leaves are pentagonal-circular or triangular-circular; 3) The width exceeds the length by more than 10%. The leaf is pentagonal or triangular.

If the area of a leaf is considered as the sum of the areas of the individual geometrical figures (Fig. 2), it may be expressed as:

$$S = \frac{B(A-b)}{2} + \frac{h(B+a)}{2} + \frac{(a+d)(h-h)}{2} + \frac{dl}{2}. \qquad (2)$$

Knowing all the parameters of this formula, and making a certain correction for the asymmetrical character as well as the serrate character which is often observed, it is possible to calculate the leaf area.

The form of leaves of the first and third groups differs by the absence of the figure DEIJ and the parameters a and h, which coincide with d and b. The formula for the area of leaves of the first and third groups is:

$$S + \frac{B(A-b)}{2} + \frac{b(B+d)}{2} - \frac{dl}{2}. \qquad (3)$$

A comparison of the separate geometrical figures in a leaf blade shows that there is a fairly clear relationship among the linear parameters within a given leaf type. Thus, average values of the parameters of a leaf of a certain type give ration approximating those shown in Table 1.

This nearly constant relationship of the various parts of the leaf to one another makes it possible to produce almost perfectly the form of a leaf typical of its group if the width (B) and Length (A) are known.

Using these ratios as regular and constant for a large number of leaves, and expressing all linear parameters in terms of A and B, we obtain the following formulae for the areas of cucumber leaves:

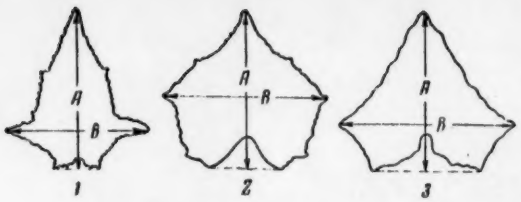

Fig. 1. Forms of leaves from one cucumber plant. 1, 2 and 3) Principle type forms; A – length; B – width.

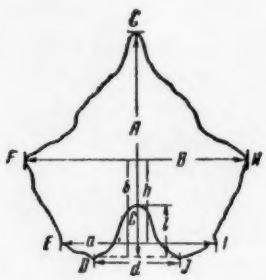

Fig. 2. Scheme of division of a leaf into separate geometrical figures for determination of the assimilation surface (explanation in text).

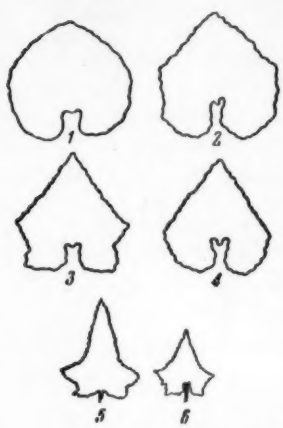

Fig. 3. Leaf forms of cucumbers occurring throughout the world according to A. I. Filov. 1) Circular; 2) pentagonal-circular; 3) pentagonal; 4) triangular-circular; 5) elongated triangular; 6) triangular.

TABLE 1

Ratio of Individual Linear Parameters in Leaves of a Given Type

Leaf type	B : a : d	A : b : e : h
1	1 : 0 : 0.33	1 : 0.25 : 0.8 : 0
2	1 : 0.7 : 0.38	1 : 0.43 : 0.22 : 0.38
3	1 : 0 : 0.65	1 : 0.29 : 0.24 : 0

for leaves of the first group $S_1 = \frac{AB + 0.06\,AB}{2}$;

for leaves of the second group $S_2 = \frac{AB + 0.19\,AB}{2}$;

and for the third group $S_3 = \frac{AB + 0.14\,AB}{2}$.

Comparison of areas obtained by the weighing method and those obtained by calculation from the formulae is made in Table 2.

A comparison of results obtained by weighing and by calculation from formulae shows that for each leaf type there are large deviations and both directions, the greatest errors occurring with leaves of the first type, which are the smallest and which often deviate from the average form. The smallest percent of deviation is found in leaves of the second type, which comprise in area the major portion of the assimilating surface.

The deviation of the total leaf area of a single plant did not exceed 5%.

According to A. I. Filov, the varieties of cucumber in the world have the following leaf forms: circular, pentagonal-circular, triangular-circular, elongated-triangular, and triangular (Fig. 3).

Comparing our classification of leaves on a single plant with Filov's classification of leaves from many varieties, it is evident that the formulae proposed may be adapted to leaves of other subspecies and varieties of cucumber (Table 3).

The proposed formulae may be applied to all leaf forms except the cirlar form. It should be emphasized that on a single plant the leaves are of different ages, and it is necessary to classify them as one of the three above-mentioned types before calculating areas. The formula for the first form type must be used in calculating the areas of young leaves, which are usually elongate in form.

TABLE 2

A Comparison of Areas (in cm^2), Obtained by Different Methods

Type of leaf blades	No. of leaf blades											
	5			10			20			30		
	1	2	% of deviation	1	2	% of deviation	1	2	% of deviation	1	2	% of deviation
1	43.57	47.63	+9.3	74.39	79.75	+7.2	153.85	163.7	+6.4	247.16	260.76	+5.5
2	2116	2279	+7.7	3776	3599	—4.7	7284	7037	—3.4	11246	11594	+3.1
3	2112	2300	+8.9	4907	4583	—6.6	10193	9612	—5.7	13505	14166	+4.9

Explanation: 1)- determination by weighing method; 2)- calculation from formulae.

TABLE 3

Adaptation of Formulae for Leaf Surface Area to Varieties in Different Subspecies and Varietal Groups

Subspecies	Varietal Group	Geographical distribution
Area calculated according to the formula for the second group $S_2 = \frac{AB + 0.19\,AB}{2}$		
(Leaf form triangular-circular, pentagonal-circular)		
1. Indo-Japanese	1. Abkhaz	USSR, China, Europe
	2. Indian	India
	3. Japanese	Japan
2. Chinese	1. Western-Chinese	Western China, Western Europe
	2. Southern Chinese	China, Western Europe
	3. Klinsk	USSR
3. Western Asiatic	1. Iranian	USSR, Iran, Afghanistan
	2. Central Asiatic	Central Asia
4. Himalayan	Himalayan	India
Area calculated according to the formula for the third group $S_3 = \frac{AB + 0.04\,AB}{2}$ (Leaf form triangular, pentagonal)		
1. Wild		India
2. Western-Chinese	Kilikii	United Arab Republic
3. European-American	1. Northern	USSR
	2. Eastern European	USSR
	3. European-American	USSR, USA, Western Europe
4. Himalayan	Bogarnaya Indian	India
5. Chinese	1. Western-Chinese	Western China, Western Europe
	2. English	Western Europe
	3. German	China, Europe, USA
6. Western Asiatic	1. Iranian	USSR, Iran, Afghanistan
	2. Astrakhanian	USSR
	3. Anatolian	Western part of Asia Minor
	4. Kilikii	United Arab Republic
7. Hermaphroditic		USA

Thus, these formulae may be used in calculating the total area of a large number of leaves (nor less than 30), i.e., when the individual deviations from typical forms do not substantially affect the overall result.

This method of assessing leaf area may find wide application in research work, since it yields quite accurate results and significantly reduces the time required.

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RESPONSE OF CORN TO SHORT DAYS IN RELATION TO PLANT AGE AND SPECTRAL COMPOSITION OF LIGHT

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It is well known that corn does not exhibit a single type of photoperiodic response [1, 2].

While very early varieties are almost neutral in their response to day length, varieties with a long growing period, mainly those from tropical and subtropical regions, have a definite short-day reaction.

The development of the plant is influenced not only by day length but also, to a significant extent, by the intensity and spectral composition of the incident light. It should be pointed out, however, that information on the effect of light with respect to intensity and spectral composition has not yet been properly evaluated. A study of these processes is of considerable theoretical and practical interest.

This investigation was designed to determine at what age the various varieties of corn are most sensitive to day length. The effect of spectral composition of light on the development of various corn varieties was determined at the same time. Experiments were performed in Berdyansk, Zaporozh region, with a collection of corn varieties from VIR and other institutions.

All plants were grown on chernozem soil, using the cultivation procedures for growing corn in the southern districts of the Ukraine. The plan of the experiment is illustrated in Table 1. According to this plan, plants of

TABLE 1

Emergence of Tassels in Different Corn Varieties Grown on a Short Day Beginning at a Certain Age (experiment performed in 1956, sowing on May 3, sprouting on May 10)

Variety and VIR catalogue No.	No. of days after sprouting before plants were exposed to 10-hour days							
	1	3		6		9	12	
	No. of days of exposure to 10-hour days							
	2	4	6	7	9	15	20	30
Beloyar millet	16.VI							17.VI
K-2740, DVK	18.VI							18.VI
Early pearl	27.VI							26.VI
K-3408, Western Chinese	26.VI							25.VI
North Dakota		29.VI						28.VI
Rice-corn 645		26.VI						27.VI
Dneperpetrovsk			29.VI					29.VI
Grushev			29.VI					29.VI
Partisan			30.VI					30.VI
K-9799, Italian				29.VI				28.VI
K-6663, Arizona					3.VII			1.VII
K-3523					5.VII			2.VII
Pride of Saline						1.VII		30.VI
K-2078, Mexico							1.VII	30.VI
Teosinte							2.VII	30.VI

TABLE 2

Effect of the Spectral Composition of Light on Corn Growth and Development (experiment performed in 1954, sowing April 29, sprouting May 9)

Variety and VIR catalogue No.	Days from sprouting to tassel emergence			Ht of plant, cm											
		10-hour day		ordinary day				10-hour day exposure, 7 a.m. to 5 p.m.				morning-evening exposure*			
	ordinary day	exposure, 7 a.m. to 5 p.m.	morning-evening exposure*	10.VI	14.VI	17.VII	23.VIII	10.VI	14.VI	17.VII	23.VIII	10.VI	14.VI	17.VII	23.VIII
Beloyar millet	37	37	44	65	72	140	140	31	40	90	130	41	46	123	130
Bezenchuk	50	43	63	62	69	160	175	45	51	117	117	29	39	120	190
K-2740, DVK	44	42	49—50	58	69	178	178	62	71	200	200	43	52	193	197
K-117116, Primor'e region	44	43	49—50	74	83	226	226	73	85	145	145	59	65	185	189
Grushev	55	48	67	53	78	234	234	47	62	171	174	32	46	125	225
K-10733, Western Chinese	51	47	49—50	63	67	200	200	51	55	153	158	29	58	155	170
K-5048, Uzbekistan	62	57	70	46	73	210	272	45	57	70		49	38	135	230
Gangan	54	48	75	57	80	220	220	44	62	180	182	21	28	105	150
Pride of Saline	74	59	87	52	71	320	320	57	62	230	230	41	47	131	252
Teosinte	—	41	93	21	65	210	270	27	31	125	150	21	28	—	—

*After 40 days plants were grown on an ordinary day.

each variety received different numbers of short days (from 1 to 20) beginning at various ages (from the day of sprouting, the 3rd, 6th, 9th and 12th day). The reaction of a given variety to a given number of short days applied at a given age was determined in comparison with a control. In the control treatment, plants of each variety were grown on a short (10-hour) day for 30 days. If the date of emergence of the tassel on plants grown for 30 days on short days coincided (with minor deviations) with the date of tassel emergence in a variety which had received the minimum number of short days for the given age, then we considered that plants of the given age had reacted to the photoperiod.

The data in Table 1 show that in corn a sensitivity to day length arises at different ages in different varieties. Early varieties respond to a change in day length from the time of sprouting, while varieties which mature later respond at later stages of development (on the 5th-12th day). In early varieties, the most rapid development of tassels occurred with a 1-to 3-day exposure to short days; for varieties maturing somewhat later this occurred after a 6-to 9-day exposure and for late varieties, after a 10-to 20-day exposure.

Corn is especially sensitive to the spectral composition of light and also to its intensity. As Razumov [3] and other workers [1, 4] have shown, red light affects plant development most strongly. According to published information [4], the exposure of plants (wheat, corn and others) to light during the morning and evening hours only (i.e., at the time when the light is poor in ultraviolet wavelengths) inhibits their development.

In this study, the effect of spectral composition of light on plant development in various corn varieties was determined under under 10-hour days; one group of plants was exposed to light from dawn to 9 a. m. and from 4:30 p. m. to dusk. The other group was exposed to light from 7 a. m. to 5 p. m. Thus, the two groups of plants in each variety received days of equal length (10 hours), but the light varied in intensity and spectral composition. In the morning and evening hours the red and yellow components of white light are predominant, and in the intervening hours the blue, blue-violet and ultraviolet components are predominant. The effect of a given treatment was clearly manifested in the growth and development of the corn.

As Table 2 shows, formation of tassels and growth of leaves and stems is considerably slower in plants exposed to light in the morning and evening hours than in those which were exposed in the intervening period.

In light poor in the short wavelengths, all varieties, with the exception of the extremely early ones (Beloyar millet; K-2740, DVK; K-11716; Primor'e region; K-10733, Western China; and others) remained at the 5-to 8 leaf stage for a long time (about 40 days), while the control plants during this period had begun to form abundant tassels. In plants exposed to light during the morning and evening hours, the leaves and stems had a light yellow color. After 40 days these plants were grown on an ordinary day, since prolongation of this regime caused the death of many varieties. From this time, growth processes were stimulated and at the end of the growing period the plants in this group had exceeded in height both the plants which had been grown on a short day and on an ordinary day (Table 2).

The response to the spectral composition of light varies somewhat from variety to variety. Early varieties (Beloyar millet; K-2740, DVK; and others) proved to be less sensitive to a deficiency in the short wavelengths. In these varieties, tassels emerged in plants grown in the morning and evening hours eight-nine days later than in plants grown in the intervening hours; in varieties maturing somewhat later and in late varieties, they emerged 20-40 days later.

It is not difficult to see that the response to spectral composition of light of a given variety reflects light conditions prevailing at the latitude at which the variety was developed.

Our investigations establish that the existence of a photoperiodic reaction in corn depends on plant age. In very early varieties it is manifested from the time of sprouting, and in later varieties, from the five-to nine-leaf stage.

Corn requires a certain light regime for flowering which consists of from 0 to 20 short days, depending on the variety.

Passage through the light stage normally occurs at a definite intensity and spectral composition. The absence or deficiency of the short wavelengths strongly inhibits growth and development in corn, especially in late and very late varieties. Very early varieties are able to develop even under morning–evening illumination.

LITERATURE CITED

[1] G. A. Samygin, Tr. Inst. Fiziol. Rastenii im. K. A. Timiryazeva (Izd. AN SSSR) 3, 2 (1946).

[2] V. F. Portyanko, Doklady Akad. Nauk SSSR 24, 5, 1077 (1952).

[3] V. I. Razumov, Tr. po Priklad Bot., Genet. i Selektsii 27, 5 (1933).

[4] F. A. Kuperman, E. I. Rzhanova, T. A. Kapitonova, A. P. Zhakinova, N. S. Lyubivaya and B. M. Lyubivyi, Vestnik Moskov. Univ. 9, 121 (1955).

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THE INTRODUCTION OF COMPLETED RESEARCH INTO AGRICULTURE

ACCELERATED PROPAGATION OF SEEDS OF NEW VARIETIES AND BIENNIAL HYBRIDS BY GRAFTING

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Biennial plants – sugar and table beets, cabbage, carrots, andothers–produce seeds in their second year. In seed-selection studies and in practice it is frequently necessary to increase the seed of perspective new varieties and hybrids rapidly.

Within the last ten-year period during studies on the ontogeny of biennial seedlings we and other investigators showed that when these seedlings are grafted on annuals or on seed-producing plants of their own variety, one could obtain seeds two-three months after grafting. Furthermore, it is not necessary to await the two-month growth as is required during vernalization; grafts are effective with 10-to15-day-old- growths well as more mature plants. The grafting method makes it possible to avoid the expenditure of winter storage and the loss of plants from diseases in the winter period. Grafts of unvernalized carrot and beet seedlings started to bloom equally well on the natural seed stalks as well as on the annual plants. Unvernalized cabbage seedlings did not bloom when grafted on the natural seed stalks; they bolted only when grafted on to annual plants—summer rape, mustard, and others.

On the basis of our greenhouse and field experiments over many years, and also a study of the variability of seed progeny after grafting, we concluded that when seedlings are grafted on the same or closely related highly productive varieties, there is no basis to fear negative variability. It also appeared that in case of remote in interspecies and intergenus grafts of biennials onto annuals (carrots on dill, cabbage on mustard, rape, etc.) that no disadvantageous variabilities were observed in the first generation.*

The grafting method and future cultivation of the seeds is described below.

Unvernalized cabbage seedlings started to bloom equally well when they were grafted on either mustard or rape. However, on mustard, an early maturing plant, the leaves become yellow early and soon abscise, consequently the grafted seedlings produced weak inflorescences, and the late maturing varieties of cabbage barely bloomed at all. The plants of summer rape were better in this respect; its leaves are sturdier and live longer. Seedlings of early maturing cabbage varieties (type number Pervyi), when grafted on mustard or summer rape, bolted within 27-30 days after grafting, but the late maturing varieties of cabbage of type Amager bolted 45-50 days after grafting. Bolted plants produced mature seed after a month, i.e., 2-2.5 months after grafting.

Wild plants of summer rape were planted in wide rows 40-50 cm apart (in order to facilitate grafting and caring for them) under field conditions during the usual spring period at the end of April to the beginning of May on the Moscow plain; in a greenhouse or hotbed this could be done at any season of the year. About a month after germination, in the middle of June, rape bolted and bloomed. During this time, when the succulent stems reach a height of 30-40 cm , they are suitable for the grafting of cabbage. The cabbage should be sown about 20 days before grafting (end of May).

*The basis of this method was reported in papers published by the author in the journals: Fiziol. Rastenii, No. 1, 1955; Vestnik sel'skokhozyaistvennoi nauki, No. 12, 1958.

In the Moscow region early maturing varieties of cabbage can be grafted until the 10th to 15th of July, and the late maturing varieties until the 1st of July. The seeds are unable to mature when grafting occurs later than this date. The grafts should be made in the evening after 2-4p.m.,and it is best to choose cloudy, cool, rainy days. Before grafting,the unnecessary side shoots on the biennials should be removed; otherwise they grow rapidly, intercept all the nutrients, and as a consequence the grafts die. The grafts are made in a slit on the main stem and on vigorous side shoots. The grafting site is tied with soft strings, raffia, and other materials, and wrapped in moist cotton. If it is possible the plot should be watered, and the cotton should be moistened with water from a funnel twice a day. All the axillary shoots should be removed. The cotton and string can be removed about two weeks after the grafting. After the graft has become established the leaves should be removed as they appear up until the buds appear on the grafted seedlings.

Under such conditions near Moscow no less than 50% of the grafted plants grew, bolted, and produced normal seeds in the beginning of September.

Grafts of carrot and beet seedlings were made on stocks of varieties which were related in respect to high productivity and other characteristics. In order to do this vernalized (from storage) fleshy roots were planted at field conditions in the usual way, if possible before the end of April in Moscow. In early June the plants began to bolt; at this time, when the stems (flower stalks) reach a height of 30-40 cm and become succulent, they are suitable to receive seedling grafts. The material to be grafted should be sown 20 days before grafting in the case of beet, and 30 days in the case of carrot. Before grafting,all the small side shoots on the stock plants should be removed and the large ones left for grafting. The grafting conditions and the later treatment are the same as for the cabbage grafts.

On the Moscow plain beet grafts and especially carrot grafts should be made no later than the 15th-20th of June; otherwise their seeds do not ripen. The leaves can be left on the scions. Sometimes the leaves die rapidly on biennial carrot and beet plants. However, one need not be concerned with this since these fleshy roots have large food reserves, at the expense of which the scions grow, bolt, and form seeds even when no more leaves are present on the stock.

Spring sowings of dill can also be used as stocks for carret grafting; the seed progeny did not exhibit any disadvantageous changes. However, this is possible only in the southern regions.

In 1959 this method was checked at the experiment stations.

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METHODS

AN APPARATUS CONTAINING RUBBER CHAMBERS FOR PHOTOSYNTHESIS EXPERIMENTS UTILIZING CIRCULATING RADIOACTIVE CARBON DIOXIDE

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In setting up experiments on photosynthesis and dark absorption of radioactive carbon dioxide it is frequently necessary to renew the gas mixture in the photosynthesis chamber. This is accomplished by using various kinds of pumps. In spite of the practical importance of an exact description of the apparatus and the technical data about the pumps which are useable (i.e., primarily safe) for investigations with radioactive gas, the literature usually circumvents the structural aspects, or else only gives an external view of the apparatus. Bearing in mind the interest of many laboratories in pumps which are usable in investigations with radioactive gases this paper contains a description of an apparatus which is easy to assemble from common rubber chambers (for waste balls) and a Komovskii pump.

This apparatus appeared to be very useful and reliable when tested repeatedly in experiments utilizing radioactive carbon dioxide gas.

A diagram of the principle of the apparatus is given in Fig. 1 (for simplicity all the details which are not absolutely necessary to illustrate the principle of the activity of the apparatus are omitted in the diagram). It consists of two accessory rubber chambers (A_1 and A_2) and one or several working chambers (C) where photosynthesis occurs, joined together with rubber tubes with screw clamps (a_1 and a_2). Each of the rubber chambers is enclosed in a round-bottomed flask, or in a sufficiently durable wide-mouthed bottle (B_1 and B_2) of approximately the correct volume, which can be attached to a Komovskii pump or else connected to the atmosphere with the aid of three-way stopcocks b_1 and b_2.

In order to check the working procedure of the apparatus, let us assume, for example, that the gas is first found in chamber A_1 and it must be passed through the working chamber C into chamber A_2. In order to do this flask B_1 is connected to the atmosphere by b_1 and at the same time is disconnected from the pump, clamp a_1 is opened and clamp a_2 is closed; flask B_2 is connected with the pump, disconnected from the atmosphere and evacuated. The pressure within the rubber chamber is approximately equal* to the pressure of the surrounding air, therefore as the flask is evacuated the pressure in the corresponding rubber chamber also decreases, whereas the pressure in the remaining part of the apparatus is equal to that of the atmosphere. Now, if clamp a_2 is opened, the gas in chamber A_1 will flow into chamber A_2 because of the difference in pressure and thus replace the gas mixture in the working chamber. The rate of flow of the gas can be regulated as desired by the screw clamp a_2. When chamber A_1 has been completely emptied of gas, as indicated by the constriction of the chamber, the process is reversed; flask B_2 is connected with the atmosphere, and flask B_1 to the pump, etc. Sometimes conditions of the experiment require that the gas pass through the working chamber in only one direction, for example, from left to right. In this case it is necessary to connect chamber A_1 and A_2 with a supplementary tube provided with a clamp a_3 as shown by the dotted line in Fig. 1.

*The pressure of the gas within the rubber chamber is greater than the pressure of the surrounding area by the amount of the actual pressure exerted by the chamber which occurs as the result of the stretching of the rubber. The actual pressure of the chamber can be disregarded when the chamber is not stretched very much.

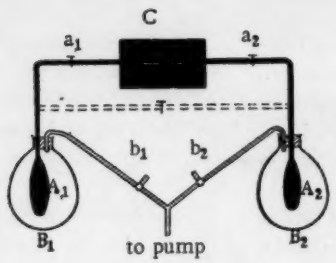

Fig. 1. A diagram of the principle of the apparatus (explained in the text).

Figure 2 consists of a complete diagram of the apparatus which we used in our work, and Fig. 3 shows an external view. This apparatus as compared with the one illustrating the principle of the procedure is provided with one more rubber chamber A_3 (the significance of this will become clear from the following). In addition the following were added to the apparatus: reactor R for obtaining gaseous $C^{14}O_2$, manometer M of the open type, mercury indicator I which serves as a visual indicator of gas movement, and, finally, a carbon dioxide absorbent P, and a β-ray counter S with a miniature circulating chamber of plexiglas. The last items are attached in parallel whereby they can be connected to or disconnected from the system either separately or together using the three-way stopcocks c_5 and c_6 (Fig. 2).

The procedure for using the apparatus is as follows. The plant material is placed into the chamber in which photosynthesis is to occur; the chambers are closed, evacuated* to about 80-100 mm of mercury and checked for leaks with a manometer. All the rubber chambers are disconnected from the working ones with stopcock c_3 and a_2, a vacuum is created in chamber A_1 and A_2 as the result of air being removed from flasks B_1 and B_2 and radioactive carbon dioxide is introduced into the apparatus.

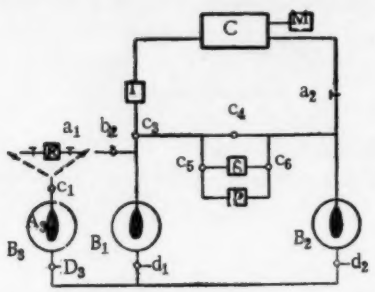

Fig. 2. Complete diagram of the apparatus (explained in the text).

In order to do this, the reactor is connected to chamber A_1 by clamp a_1 and stopcock c_2, after this acid containing a dissolved radioactive salt of carbon dioxide is gradually introduced. (We used hot concentrated sulfuric acid which we introduced into the reactor in great abundance). One to one-and-a-half liters of air are passed through the reactor in order to completely remove all traces of radioactive carbon dioxide from the reactor, and to bring the concentration of CO_2 in chamber A_1 to the necessary value—about 3%. By means of stopcock c_2 the reactor is then disconnected from the apparatus, vessel B_1 is connected to the atmosphere and part of the gas in chamber A_1 is directed into the working chambers by carefully turning the three-way stopcock c_3. When the pressure in the working chambers has increased almost to atmospheric pressure, clamp a_2 is opened and the gas begins to enter chamber A_2 as we described in the diagram illustrating the principle. In order to get a better circulation of the gas we preferred to have it move in one direction, therefore the gas from chamber A_2 was rapidly transferred to chamber A_1 while the working chambers were disconnected and stopcock c_4 was open.

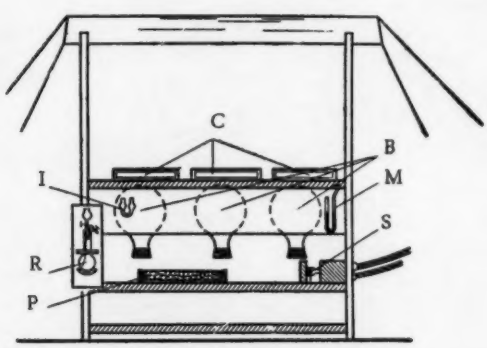

Fig. 3. Front view of apparatus (the connecting tubes are omitted in the diagram): C) photosynthesis chamber; B) flask with rubber chamber; I) gas movement indicator; S) counter with circulating chamber and container BGS.

After the exchange the content of radioactivity in the system was measured by passing the gas current through the counting chamber S. Stopcock c_4 is closed during this, and stopcocks c_5 and c_6 are also closed when the absorbent is disconnected from the apparatus. If the radioactivity is still quite high, the main part of it is transferred to chamber A_3 which is attached in place of the reactor and serves as a reservoir for the unused radioactive material until the next experiment. (It is understood that in such a case the reactor may not be needed).

*To avoid damaging the counter during evacuation, it should be disconnected from the remainder of the apparatus.

The radioactivity remaining in the working chamber is absorbed by passing the gas through an alkaline absorbent P.

We installed this apparatus on a wooden rack (see Fig. 3) covered with a white oil paint and provided it with an electrical outlet for illumination at night and for using the counter. The rack had three shelves and a plastic cover to protect it from the rain. The photosynthesis chambers occupied the entire upper shelf. Three round-bottomed flasks of approximately three liters each, and each with a rubber chamber, were attached neck down below the shelf. On the second shelf were attached the connecting tubes, stopcocks, carbon dioxide absorbent, and finally the circulating chamber with the counter and the removable chamber BGS. The counting apparatus itself of type B-2 stood on a separate table. The lower shelf contained the necessary reagents and instruments. The manometer, indicator, and reactor were attached to the side of the rack for convenience; the Komovskii pumpstood on the gound on this same side.

We would like to say a few words about the gass movement indicator I. It was in the form of a U-tube, and was made from two potassium chloride tubes. Its narrow neck contained some mercury which, first of all, gives some resistance to the circulating gas, and secondly, permits one to observe gas movement immediately.

In conclusion we will add that the use of rubber chambers makes it possible to renew the radioactive gas mixture in the working chamber either continuously or periodically with complete safety since the pump itself is completely separated from the radioactive gas. In addition, all the conveniences of the Komovskii pump are retained: large power, the possibility of using the rubber chambers to regulate the pressure in the working chambers, and the ability to retain the main part of the unused activity for future experiments.

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A METHOD FOR DETERMINING THE OSMOTIC PRESSURE OF CELL SAP USING COLLODION SACKS

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In the resolutions proposed in 1955 in the Moscow All-Union report concerning the problem of "Biological bases of irrigation farming" there was an indication of the need for developing and widely introducing into agricultural procedures simple physiological methods for determining the water balance of the plants in order that the watering of agricultural crops be well timed.

One of the most sensitive objective physiological indicators of the water balance of the plants is the osmotic pressure of the cell sap [1-5].

However, the existing plant physiological methods of determining osmotic pressure of cell sap (plasmolytic, cryoscopic, Bardzhera-Rasta) are intricate to use and, consequently, are seldom used under field conditions.

In recent years simpler methods for determining the concentration of the cell sap have been proposed (refractometer [6], "struek" [7, 8]), which enable one to calculate the osmotic pressure. However, they do not always yield figures which correspond to results obtained for the osmotic pressure of cell sap as determined by other methods. In particular, both of these methods (as our studies showed, see table), as compared with other method yield an increased value for the osmotic pressure of cell sap in plants with large quantities of colloids.

While studying the water relation of cotton in 1952, we were confronted by the necessity of finding a rapid and accurate method for determining the osmotic pressure of cell sap which would be suitable to use in making determinations at field conditions.

As the basis for such a method we proposed comparing the osmotic pressure of two solutions separated from each other by a semipermeable membrane. One of the solutions being cell sap, the other a sucrose solution of a known osmotic pressure.

In substance it consists of selecting a sucrose solution with an osmotic pressure which corresponds to that of the cell sap. In practice it is not always possible to select the exact sucrose solution concentration which would correspond to the osmotic pressure of the cell sap. In such cases it is adequate to select solutions of the two next higher concentrations of sucrose where a diffusion of water still occurs from the cell sap in one of the samples, but not in the other sample where on the contrary, the water from the sucrose solution begins to diffuse into the cell sap which is enclosed in the semipermeable membrane. The value for the osmotic pressure of the cell sap is then conventionally taken as the simple arithmetic mean of the sum of the osmotic pressure of the given sucrose solutions.

The semipermeable membrane used in this method is a collodion sack which can be easily and quickly obtained.

At the beginning of the experiment a wide range of sucrose concentrations are used [8, 10]; this range can be narrowed down later depending on the particular experiment.

Once the approximate limits of the concentration of the sucrose solutions in which the osmotic pressure of the cell sap lies are determined, the accuracy of the determinations can be increased by decreasing the diversity

TABLE

Osmotic Pressure of Cell Sap (in atmos) as Determined by Various Methods (1958 experiment)

Experimental material	Method				Experimental material	Method			
	cryoscopic	struek	refracto-meter	collodion bag		cryoscopic	struek	refracto-meter	collodion bag
Lemon, Genoa	13.25	14.60	14.00	13.20	Lemon, Lisbon	11.17	12.30	12.10	11.21

between the concentrations of the solutions. The method is sufficiently sensitive, and can be used to consider differences in osmotic pressures of 0.1 atmos, which corresponds to a 0.004 molar change in the concentration of the sucrose solution.

The concentration of the sucrose solution is determined by a refractometer. However, one can also confine himself to a visual evaluation of the osmotic pressure of the cell sap in the plant on the basis of observed changes in the volume of cell sap in the collodion sack. We made use of tables containing the osmotic pressure of sucrose solution contained in the paper by Maximov and Petinov [9] to calculate the osmotic pressure of the cell sap.

For making determinations at field conditions the sucrose solutions were poured into the sample tubes before going out into the field, and in order to reduce the warming up of the samples, the cardboard box in which they were kept was placed into a plywood container either painted white or covered with white paper.

Before the cell sap was expressed (with a hand press or tongs) in the field, the leaves were first carefully wiped with moist (in order to remove the dust) and then dry gauze. Using a pipette an exact number of milliliter (no less than 0.2 ml) of cell sap were distributed among the collodion sacks. Before the work was begun these had been removed from a container with distilled water, dried carefully with filter paper, and straightened by inflating them with air. The collodion sacks with the cell sap were quickly immersed into the tubes with sucrose solutions and were immediately recovered with rubber stoppers. Following this the chamber was transferred to the laboratory, and after 1-2 hours the determination was completed.

Above, in the table, are presented for comparison the results of determinations of the osmotic pressure of the cell sap in lemon leaves obtained by our method, as well as by three well-known methods used in plant physiology.

A study of the data given in the table shows that the method which we propose for determining the osmotic pressure of cell sap in plants in all of its simplicity yields results which correspond in accuracy to those obtained by the difficult cryoscopic method.

SUMMARY

A simple but comparatively accurate method for determining the osmotic pressure of the cell sap of plants using collodion sacks is described; the method can be used under field conditions.

LITERATURE CITED

[1] G. O. Val'ter, Collection of 25 Years of Work of B. A. Keller [in Russian] (Voronezh, 1932).

[2] P. A. Genkel', Tr. Inst. Fiziol. Rastenii im. K. A. Timiryazeva AN SSSR 5, No. 1 (1946).

[3] N. A. Maksimov, A Collection of Papers concerning Drought Resistance and Winter Hardiness of Plants 1 [in Russian] (Izd. AN SSSR, 1952).

[4] B. A. Keller, Transactions of the B. A. Keller Laboratory of Evolutionary Plant Ecology 3 [in Russian] (Izd. AN SSSR, 1952).

[5] N. S. Petinov, Fiziol. Rastenii 1, 81 (1954).

[6] M. G. Lobov, Doklady Akad. Nauk. SSSR 66, 2, 277 (1949).

[7] V. M. Artsikhovskii, Planta 14, 3-4 (1931).

[8] V. S. Shardakov, Theses of Reports on Plant Physiology [in Russian] (Moscow, 1940).

[9] N. A. Maksimov and N. S. Petinov, Doklady Akad. Nauk SSSR 62, 4, 537 (1948).

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SUCCESSES IN AGRICULTURE AND BIOLOGICAL SCIENCE IN THE CHINESE PEOPLES' REPUBLIC

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Many interesting articles and a number of individual, interesting books have already been written about the immense achievements of the great Chinese people, who are building socialism at a vigorous rate. However, only a short time passes and we are witnesses of new successes in the development of the various branches of the national economy and culture of the Chinese People's Republic, which the Soviet people note with great gladness. I was one of these witnesses in 1959 while living there on a scientific detail at the invitation of the Academy of Sciences of CPR *.

1. What I Visited and Saw

In the three months of my visit in the CPR, I was in Peking, Tientsin, Sian, Ugun, Shanghai, Nanking, Hangchow, and Kuangjou (Canton), where I became acquainted with their curiosities and, more important, with the scientific-research work of the many universities, institutes, and scientific institutes. I visited several people's communes: First of July, near Shanghai; Sikhu, near Hangchow; Pang-Yü and Sing-hao, not far from Canton. I saw the enormous irrigation canal "Věikhé" in Shensi province, which stretches for 336 km and makes possible the irrigation of 113,000 hectares of land, and the large Shisyanlinsk reservoir with water reserves of 60 million cubic meters with an impressive dam 626 m long and 28 m high.

In Peking I became acquainted with the All-China Agricultural Exhibition of 1958 and with the All-China Exhibition of Industry and Transportation, which significantly broadened my idea of the tempo and character of the development of the CPR's national economy.

With the first days of a visit in this interesting country, one already experiences the immediate and indelible strength of its different forms, the novelty and richness of the impressions when looking over the great yeards with their fantastically lovely parks and lakes, the numerous monuments, the wooden temples and pagodas, museums, city parks, etc.

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* The program of my stay in the CPR was approved by the Institute of Plant Physiology of the CPR Academy of Sciences (Shanghai), which directed me. I was shown the most cordial reception and given all conditions for fulfilling my program by the direction of this Institute (director, Prof. Lo Tsung-lo, vice-director, In Hung-chang and Chao-i; and the party organizer, T'ang Ten-ting).

I also received invariable and friendly greetings from the directors and workers of those scientific institutes, higher educational institutions and people's communes which I was able to visit.

My interpreter, Fang-I-sung, a worker in the Institute of Plant Physiology of the CPR Academy of Sciences, and also the interpreters of my lectures, Chao Shih-hsü and Wang-Wan li, were of great help during the entire period of my stay in the CPR. I extend my very heartfelt thanks and recognition to them.

However, the admirable Chinese people gladden and delight one most of all. When you go out at night, look about and you will see not only large, but also numerous small, homemade, active blast furnaces, so that it seems that the entire country is aglow with bonfires;this country is smelting pig iron and steel. Day and night along the railroad, thousands of people are working, chiefly untiring young people happy with life; they are building a second road or laying a new line. They are doing this at the present time without complete mechanization, dragging the earth in baskets on yokes and packing it by hand. Stones are also handled in the baskets. The given rhythm is not interrupted a single time; the people do not stop their movement. There are no shouts and no noise and it is hard to believe that these people are carrying out heavy physical work so beautifully. Hundreds of thousands of people are building dams, digging canals, building hydroelectric stations, or are occupied simply with field work. The city is literally boiling with great, tense work. When you see all of this, you believe that under the direction of their Communist Party and the government, the Chinese people are, of course, building socialism. "Bigger, faster, better, and more economically" is the basic motto of the development of the national economy in China. Industry and agriculture are developing simultaneously in this way. The slogan, "walk on two legs", which is commonly used in the CPR, is being accomplished.

2. The Great Success – "the Big Leap" – in 1958 in Agriculture in the CPR

The past year, 1958, is, as we all know, the year of the sudden climb, the year of the "big leap", for the CPR in all areas of the national economy and culture.

The great successes noted in the agriculture of the CPR are unprecedented. The past year of 1958 appeared to be very unfavorable for China as far as meteorological conditions were concerned. Drought hit more than 30 million hectares of arable land, almost a fourth of the entire area. Also, about 7 million hectares of field suffered from an excess of rain. In spite of this, unusually high yields were obtained. The average yield of grain crops for the country reached 21.33 centners per acre and in five procinces, Chiangsu, Anhuo, Hupei, Szuch'wan and Hengan, rice yields reached 375 centners per hectare. Record yields were obtained on small areas (0.15-0.30 hectare), which, however, are not so small in the total (they make up one tenth of the entire area for grains). It is even hard to imagine the yields: 549 centners of winter wheat per hectare, 642 centners of summer wheat, 2771.7 centners of early ripening rice, 2654.4 centners of corn, 2022 centners of peanuts, 90 centners of rape, 7978 centners of potatoes, 336.2 centners of tea (dry weight), etc. Such yields received the symbolic name of "sputniks".

The yield of food crops as an average per hectare increased 35% in 1958 as compared with 1957 and was twice as high as in 1949. Thus, the total collection of grain increased from 185 million tons in 1957 to 250 million tons in 1958; cotton-fiber increased from 1.64 million tons to 2.1 million tons, that is, there was 28% more than in 1957.

The Chinese progressive farms in the people's communes obtained these yields as a result of the use of a completely new system of agricultural methods, the so-called "eight basic positions of agriculture". The most important of these are; 1) the effective use of water resources, including,mainly,irrigation, 2) deep plowing, 3) the layered application of large doses of organic and then mineral fertilizers, and 4) heavy sowing with variety seeds.

The development of irrigation and the scope of irrigation construction in the entire country can be judged by the fact that for 1958 alone, the area of irrigated land increased by 32 million hectares. In other words, for only the year of the "big leap", more than twice as much land was irrigated than in any other year in all of the preceding history of agriculture in the Chinese Peoples'Republic. The irrigated area at the present time approaches 68.6 million hectares, or more than 60% of all of the workable land of the country. More than one-third of all the irrigated land in the world can be found in the Chinese Peoples' Republic, and, based on this indicator, it is in first place in the world.

The deep plowing of the soil is of primary importance, and this is fully substantiated. Deep treatment increases the capacity of the loose layer of soil, improves the conditions of aeration in the deep soil horizon, makes it possible to apply larger doses of fertilizers, aids in the good development and deeper penetration of the root system, and finally is an effective means of fighting weeds and disease. In addition to this, stems are more stable with deep treatment of the soil, and this prevents breakage of the plants to a significant degree.

The deep treatment, in which the upper layers are cultivated and the lower layers are broken without being mixed, must be done, according to the Chinese progressive farmers and scientists, in the range from 33 to 66 cm; it was carried out to more than 99 cm on experimental plots with record yields, and in individual cases, even up to 200 cm. In 1958 deep plowing was done on an area of 8 million hectares in all provinces of the Chinese Peoples'Republic for the period of spring and summer planting; from autumn to the end of the same year, it was carried out on an area of approximately 53.3 million hectares, which makes up more than three fourths of the entire planted area of the country.

The layered application of large doses of organic fertilizer is called upon to play an exceptionally important role. Thus, in the province of Henang in the Sieh-p'eng district on the Huo-p'eng cooperative, where a yield of grain of winter wheat of 549 centners/hectare was obtained, the following quantities of fertilizer were applied per hectare: 900 T manure-compost mixture, 375 T raw compost, 300 T of feces, 0.37 T of ammonium sulfate, 3.75 T of ash and 0.15 T of superphosphate. Similar examples could also be given gor other crops.

The application of organic fertilizers in connection with deep plowing of the soil, as is well known, not only supplies the plants with nutrient substances for a longer time, but also sharply improves the physical qualities of the soil and also makes it possible to regulate the water and temperature regime of the soil, retain moisture and fertilizer and reduce the quantity of water necessary for irrigating the fields as a result of an increase in the moisture capacity of the soil. Organic fertilizers strengthen the action of available microorganisms in the soil and make favorable conditions for plants to obtain abundant nutrient substances. Besides this, a number of substances with high physiological activity are generated with the decomposition of the organic substances, for example, auxins, vitamins, antibiotics, and also humic acid, all of which stimulate the growth of plants. However, simultaneously with the organic fertilizers, the Chinese farmer also widely uses the application of chemical and bacterial fertilizers.

Earlier many experiments were carried out on the basis of old theory. Since they were based on one factor, they led to the normal conclusion that there was no excuse for thick sowing. For agricultural crops under such planting, they consider that small spikes and weak stems are formed, as a result of which they are easily broken and, as a result of this, little grain is obtained. The idea that on one hectare there could not be more than 6 million spikes of wheat, 150 thousand sets of rice, 37.5 thousand corn plants, etc. was strengthened. High yields in China refute this theory. They show that on one hectare, one may grow 21-22.5 million wheat spikes and more than 1.5 million rice sets. The brush from this spike is not any smaller, the amount of grain is not decreased, and the stem does not break. The harvests of grain did not fall, but, instead, increased. A new theory on thick sowing of a high degree is already constituted on the basis of thick sowing in conjunction with deep plowing and layered application of large doses of organic (basic) and mineral fertilizers. Concrete measures have been developed, such as an increase in the quantity of sowed seeds, a reduction on the area between rows, crossed planting, uniform sowing of seeds, etc.

The use of the new system of "eight agricultural rules" made it possible to overcome the old opinions in general on the nature of plants and open almost an unlimited possibility for the increase of the productivity of plants. Originally it was really thought, for example, that wheat was a low-yielding crop and that the harvest from it could not exceed 75 centners per hectare; otherwise, the wheat stems would break. It was noted in literature that even with a harvest of 50-55 centners per hectare, the stems were already breaking. However, with deep plowing, thick sowing, and the layered application of large quantities of fertilizers, record yields of wheat from one hectare, as Chinese progressive farmers showed, reach 525-600 centners per hectare and the plants still do not break.

What concrete system of agricultural methods was used by the Chinese farmers as a result of which such high yields were obtained in 1958? The answer to this question to a sufficiently accurate degree can be given by the material of P.M. Balev, published in 1959 in the journal "Udobreniya i urozhai", No 2. We would only like to give some supplementary data. The agricultural measures used for winter wheat in the educational section if the Academy of Agricultural Sciences near Peking (at present, a peoples' commune) for example, are of interest.

Green fertilizer (a legume plant) and organic fertilizer, 150 T of manure, are plowed the first time to a depth of 30 cm. Then a second plowing to a depth of 50 cm is carried out with the addition of 10 T of manure, and finally, the third plowing to a depth of 30 cm with the application of 5 T of manure. The norm for sowing

is 525 kg/hectare instead of the usual 120-150 kg/ hectare. In the winter in December an additional 10 T of manure is applied with the goal of increasing the resistance to cold of the plants. In early spring a feeding with mineral fertilizers is used: 30 kg/hectare of ammonium sulfate, 300 kg/hectare of superphosphate, and 1500 kg/hectare of ash. During the phase of stem formation irrigation is used and fertilizer is applied: 75 kg/hectare of ammonium sulfate, 37.5 kg/hectare of superphosphate, and 1500 kg/hectare of ash. During the period of flower formation, irrigation and the same fertilizers as in the phase of stem formation are again used, but only a half-dose of the latter was applied. During the phase of ripening of the grain, four applications of foliar feeding with a 4% solution of superphosphate were made. With the yellowing of the leaves, an additional nitrogen application of 150 kg and 10.5 T of feces per hectare (in solution with water) was applied.

Another agricultural technique is used in growing rice (without transplanting) in the peoples' commune "Pan-Yui" near Kuangjou (Canton).

After deep plowing to 30-33 cm, the basic fertilizer is applied (manure, compost, silt and refuse totaling 1500 T per hectare). Then the soil is thoroughly stirred and harrowed. After this, planting of a norm of 500-525 kg per hectare to a depth of 1.5-3 cm is made with 12-15 cm between rows. Planting is done on those dates on which rice seedlings are set out. When the sprouts reach a height of 12-15 cm, the field is harrowed lengthwise and crosswise for thinning and trimming the roots in order to stimulate their growth. After planting, irrigation is carried out to inundate the area with a layer of water 2-3 cm deep. As soon as possible the field is again harrowed and doubly cultivated. After 10 days the first hilling is done to a height of about 3 cm, and then on the ninth day after this, the second hilling for better development of the root system and for rich grain is done.

During the phase of flower formation, there is a "drying" in order to deepen the root system and decrease breakage of the plants. For a period of one to two weeks the soil moisture is kept in the neighborhood of 60% (nor lower) of field capacity and then irrigation inundating to a depth of 6 cm is carried out. This layer of water is maintained for a period of 20 days (up to the formation of the brush). After this a layer of water 2-3 cm deep is maintained up to the stage of wax ripeness, after which a break in the water level begins.

Simultaneously, pests and diseases of rice are fought systematically with the help of chemical preparations.

Feeding is done after the first and second cultivations: first, at the beginning of stem formation, 3-3.75 centners/hectare of superphosphate are applied, and then 7.5-11.25 centners/hectare of potassium are applied at the middle to end of stem formation for the second feeding. When the leaves yellow, or when the growth in general is slow, nitrogen fertilizer is applied in the quantity 112.5 kg/hectare (up to the phase of brush formation). When such agricultural conditions are observed, the harvest of rice is practically no different from the harvest of rice cultured from seedlings.

New agricultural methods have also been developed by the Chinese farmers and scientists for the cultivation of tea plants. Thus, in the province of Fukien, Ansi region, the "Si-ping" peoples' commune, where a record yield of tea (dry weight) of 33.62 T per hectare was obtained in 1958, used a system of measures including: 1) repeated cultivation, 2) application of large doses of organic and mineral fertilizers, 3) irrigation, and 4) protection against the first autumn frosts, etc.

Cultivation between the rows to a depth of 10-30 cm is done eight times: the first and second in March, the third, fourth, fifth, sixth and seventh in the middle of the respective months, April, May, June, July and August, and finally the eighth in the second half of September. Fertilizers are applied in the basic form on those dates when cultivation is done. The total applied per hectare: 300 T of manure, 1.29 T of ammonium sulfate, 1220.25 T of feces, more than 2.25 T of superphosphate, 1.12 T of bone meal and a large quantity of ash in a mixture with silt.

Irrigation is usually carried out in July and August during the time of drought.

Along with this, covering with wet grass (or litter) to a depth of 6-7 cm is practiced with the goal of saving the moisture in the soil.

Different methods are used to protect the tea plants from the first autumn frosts: 1) covering the bushes at night with mats of rice straw, 2) smoking by means of burning dry litter, 3) planting legume plants in the middle of the rows, etc.

Finally, the old shoots are cut off to improve the growth of the young shoots in the middle of February prior to the first harvest of tea.

From all that has been said, it is clear that the Chinese farmers have met with outstanding successes, which are revolutionary leaps of great significance for modern scientific agriculture. Such great conquest as those made in the Chinese Peoples' Republic in 1958 on the front of agricultural production are unknown in history. History does not know such absolute growth of production and tempo of improving the yields of different agricultural crops.

3. Successes of Chinese Biologists

Chinese scientists have also had great successes. They are carrying out scientific-research work with daring and initiative closely connecting it with the goal of building socialism. Because of the complete planning in the Chinese Peoples' Republic, the broad collaboration between scientific institutes, universities, and the directors and offices of production is possible.

The transformation into life of slogans introduced by the Communist Party in the area of scientific investigation led to the strong, total movement in which outstanding scientists, scientific workers, teachers, students, and a wide circle of workers are included.

The main task, to generalize scientifically and to expand the positive experiment of the "big leap," is placed before all of the scientific institutions. Of course, we must keep in mind that very little time has passed since the first beginnings of the work on this generalization. However, the Chinese scientists have, nevertheless, already obtained much interesting material in which the foremost experiment of the farmers working in the peoples' communes is scientifically generalized.

This work to a certain extent is coordinated with one of the great scientific research institutes of the Academy of Sciences of the Chinese Peoples' Republic, the Institute of Plant Physiology in Shanghai (director is Professor Lo Tsung-lo. The Institute of Plant Physiology of the CPR Academy of Sciences serves as a broad coordinator in these investigations with the many institutes and also with the peoples' communes: with the Institute of Soil Science in Nanking, with the Institute of Agronomy of the Academy of Agricultural Sciences in Peking, with the Botanical Institute both in Peking and in Canton, with the universities in Peking, Tiensin, Nanking and Shanghai, with the Pedagogical Institute in Shanghai, with the Agricultural Institute in Canton, and with many others.

The new tasks before the Institute, naturally, involved changes in its structure and a rearrangement of the scientific personnel.

There were five laboratories up to 1958 in the Institute of Plant Physiology of the Academy of Sciences of the CPR: 1) Physiology of the Water Regime, under the direction of Professor Lo Tsung-lo;2) Mineral Nutrition, under the direction of Professor Tang Ying-wie; 3) Plant Biochemistry, under the direction of Professor In Hung-chang; 4) Physiology of Development, under the direction of Professor Lo-Shih-wie,5) Physiology of Microorganisms, under the direction of Professor Shen Shang-chung.

In 1958 these laboratories were instead organized into the following sections: 1) Physiology of Highly Productive Crops, directed by Chao-i, 2) Physiology of Photosynthesis, directed by Professor In Hung-chang; 3) Physiology of Growth Substances, directed by Professor Lo-Shih-wie; 4) Physiology of Microorganisms, directed by Professor Shen Shang-chung;5) Treatment and Preservation of Agricultural Products, directed by lecturer Chao Tong-fang; 6) Radiobiology, directed by Professor Lo Tsung-lo.

Four groups were created for the scientific generalization of the experiment to obtain high yields of im portant agricultural and technical crops: 1) group on rice, directed by Tang Si-huang;2) group on wheat, directed by Tsing Chang-tsung; 3) group on cotton, directed by Tang Yung-wei, and 4)group on rape, directed by Shih Ching-t'ang.

To which questions should attention first be given, both by the many groups of the different scientific-research institutes and by the higher educational institutions?

The biological foundations of each crop were considered against a background of the applied new system of agricultural methods (that is, a combination of irrigation, deep plowing thick sowing, layered application of

large doses of organic and mineral fertilizers, and others). With this, such processes as the development of the root system, the energy of stem formation, the intensity of the growth and accumulation of dry substances of the aerial organs, and the formation of the fruiting organs were studied. Simultaneously the productivity of transpiration and photosynthesis, the management of metabolism, the structure of the harvest, quality of seeds and agricultural production, etc.,were determined.

It follows from these primary materials that the bold use of the new system of agricultural methods sharply changes,in a positive direction,the water and physical characteristics of the soil and its fertility, strengthens the microbiological processes, resolves the question of the uninterrupted nutrition of the plants, significantly improves the development and the activity of the root system and the plant in general, increases the productivity of transpiration and photosynthesis, and a strengthens metabolism, so that together these result in a colossal increase in yields. The generalization of this experiment is a rich achievement for Chinese scientists. It can allow them to create a new theory of superhigh yields.

Along with the generalization of the "big leap" in agriculture, the scientific institutes and universities are establishing similar experiments on their scientific farms or on peoples' communes with the application of the new achievements of biological science and, together with them, are developing the theoretical problems.

One of the important problems developed by the Institute of Plant Physiology of the Academy of Sciences of of the CPR (Professor In Hung-chang) in conjunction with the chair of plant physiology of Peking University (Professor Tank P'ieh-sun and others was thatphotosynthesis from the point of view of the knowledge of its mechanism is also connected with yields. We will not be concerned with the questions of the mechanism of photosynthesis because they have already been developed by Professor Rubin in an earlier published work*. I will dwell only on works on photosynthesis in connection with yields.

Previous investigations by Professor In Hung-chang on the question of the accumulation and distribution of assimilates in rice plants (11 varieties) showed that the largest part (2/3-3/4) of the dry substance of the head, that is, the harvest, depends on the degree of photosynthesis after flowering. The smaller part (1/3-1/4) is derived from other parts of the plants, mainly from the lower internodal stem, where the reserve substances are concentrated.

When the leaves are removed during the time of ripening of the grain, approximately 1/2 of the protein substances, and all of the sugar and starch are extracted from the stem; cellulose was also mobilized. In this manner, defoliation showed that the leaves are the main photosynthetic organs. This is the reason that removal of the leaves decreased the percent of filled grain.

In so far as the yield of grain is connected with photosynthesis, the size of the leaf surface, which reaches a maximum in the phase of spike formation and then even decreased somewhat, is especially important. A linear relationship between the area of the leaves and the harvest was found by the experiments of Professor In Hung-chang on three highly fertile fields in Anhuo province. Thus, with relationships of the size of the leaf area to the area of the ground of 11, 26 and 40, the yield respectively increased proportionally, 59, 81 and 130 T/hectare. However, the peak of assimilation is observed later than the peak of the size of the normal photosynthesizing surface. Also, according to In Hung-chang, the determination of the area of the leaves during the time of flowering can serve as a guide for the analysis of the maximum possible harvest. This, however, does not indicated that the actual assimilation is the same on all fields or for all leaves.

Fig. 1. Types of hilling. a) turtle shell; b) fish back; c) housetop; d) smooth

All that has been said has great theoretical and practical significance. Previously it was thought, and many scientists think so now, that the area of the leaves, as organs absorbing the sun's energy, did not have to be very large. It was maintained that, even under conditions of a good supply of moisture to the plants, the area

*B. A. Rubin, Uspekhi sovremennoi biologii, 46, No 4 (1958).

of the leaves could reach in the best cases 40-50 thousand square meters per hectare and that a further increase in the area of the leaves was not considered effective because of the big corresponding increase in shade. However, high yields of rice, sweet potato, rape and other crops in the CPR, and also the above-noted data of Professor In Hung-chang, show that this is not so. Conclusions on the narrow coefficient of absorption by the plants of light energy, which did not exceed 3-5%, were also noted long ago to be incorrect. This coefficient can be immeasureably higher and can approach, according to preliminary calculation, 30 and even 40%.

However, to increase the area of the leaves and still to see to it that they shade each other less is possible in general and under conditions of thick sowing in particular. This conclusion was reached by Professor In Hung-chang for rice. He suggests that those varieties of rice for which the leaves are very long and at acute angles with the stems be used for planting. Obviously, the Chinese plant breeders, working at the present time in the investigation of new, highly productive varieties of agricultural crops, including rice, are keeping this important characteristic of rice in view.

An ingenious and valuable idea was also brought out by the Chinese farmers. In order to improve the lighting of plants, for example, rape, they apply a different type of hilling with the typical Chinese names of "turtle back" (Fig. 1a), and "fish back" (Fig. 1b), "housetop" (Fig.1c), and "smooth" or "level" (Fig. 1d). It appeared that the best types of hilling to recommend are the forms "turtle back" and "fish back". On such hilling, the number of plants and the normal area of the leaves in a group are much greater when compared to the other types of hilling, and the degree of their lighting is not poorer.

Of great importance are the measures developed by the Chinese farmers and scientists for the battle with the breakage of grain and other crops. It is well known that breakage sharply reduces yields and lowers the quality of the seeds. However, how can breakage be avoided with very high doses of fertilizers, and even with thick sowing? As has already been stated often above, the Chinese farmers use a simultaneous system of measures: deep plowing, layered application of organic and mineral fertilizers in certain relations of mineral elements, and rational irrigation. The farmers generally recommend such mixtures as bone meal, superphosphate, plant ash, and sodium silicate.

A group of workers in the Institute of Plant Physiology of the Academy of Sciences of the CPR, in studying the reasons for breakage of rice, established that breakage is observed significantly less where rotted manure, which disintegrates rapidly and a large quantity of soluble nitrogenous substances is accumulated in the soil, causing profuse growth and, as a result, breakage of the rice stems.

In order to decrease the tempo of growth of the stems and thus decrease the danger of breakage, the soil moisture is temporarily reduced in the phase of flower formation to 60% of field capacity.

Finally, with the same goal, that is, reduction of the growth of stems and simultaneous strengthening of their walls to prevent breakage of the rice, physiologically active substances (2, 4-DU and others) have been successfully applied in the phase of stem and flower formation by the Department of Plant Physiology and Laboratory of Plant Physiology at Peking University (Professor Ts'ui Cheng and his scientific colleague Shao Li-mei).

In the Department of Plant Physiology and Laboratory of Plant Physiology of Peking, Teintsin, and Shanghai Universities, and others, and also at the Pedagogical Institute at Shanghai, a great deal of attention is being directed toward the biochemical bases of the storage of grain such as rice, wheat, and soy, and other crops, sweet potato, onion and garlic.

Questions of the removal and storage of the harvest in the CPR are very important in connection with the large quantity of precipitation in many provinces.

In the Institute of Plant Physiology of the CPR Academy of Sciences (Professor In Hung-chang and Chao Tung-fang, investigations have been carried out on grain storage, both in warehouses and in the laboratory, for different moisture contents of the grain and air temperature and under conditions of ventilation. Along with this, the concentration of fatty acids, protein, and vitamin B_1, the ability to sprout, and the quantity of microorganisms were studied. While testing the old method of drying wheat grain in the sun at a temperature of 40-50°C for a period of two-three days and in grain dryers, it was established that for rice, for example, at a temperature of 35° in the grain dryers, the moisture in the grain must not be higher than 13% and at 25°, not higher than 15%. Similar data were also obtained for wheat. However, the decrease in viability when wheat is stored depends on its physiological condition. Grain that has passed through a period of quiescence maintains its viability significantly longer.

Physiologically active substances are widely used to prevent sprouting of potato, onion, and garlic, as in the K. A. Timiryazev Institute of Plant Physiology of the USSR Academy of Sciences. The Chinese scientists use a preharvest spraying with the hydrazide of maleic acid for prevention of onion and garlic sprouting, and spraying with 2, 4, 5-trichlorophenoxyacetic acid can prolong the length of time for potato storage by two months. Positive results were also obtained with the action of ionized radiation.

Great care is taken in the drying of sweet potatoes, in which water is driven off very slowly. Inorder to speed up this process, sulfur dioxide and carbonyl chloride are used. The permeability of the cells is increased, as a result of which water is given off rapidly upon drying and the sweet potatoes can be stores well and their quality maintained.

Recently the Institute of Plant Physiology of the CPR Academy of Sciences at Peking and Teintsin Universities (Department of Plant Physiology—Professor Tank-P'ieh-sun and Professor Ts'ui Ch'eng) the Agricultural Institute of Northwest China (Professor Shih Szung-hang and other scientific institutions have been developing such broad and very important ideas as: the physiological bases of high productivity of agricultural plants, the physiological bases of irrigating rice, the physiological role of microelements, etc.

Deserving attention are some individually interesting facts established in the Insitute of Plant Physiology of the CPR Academy of Sciences. Thus, in studying the reasons for cotton buds falling off, it was found that one of the reasons for falling is the suppression of photosynthesis in the period of flowering and, as a result, the inadequate supply of assimilates for the fruiting organs of the cotton. The suppression or, in any case, the decrease of photosynthesis observed on shading and the decrease in leaf area leads to a decrease in the normal concentration of carbohydrates. Therefore, the low concentration of carbohydrates in the fruiting organs on the day of flowering can serve as a signal that the percent of the bolls off will be high. Consequently, increasing the normal quantity of sugars in the fruiting organs in the period of flowering by various means (fertilizer, microelements, regulated irrigation, etc.) can successfully fight the falling of buds and bolls of cotton. If the bolls have not fallen off in the period of seven days after flowering, then they will be retained until the end of ripening.

The question of the reasons for the formation of a significant number of nonfruiting pods of rape, which cause a decrease in its yield, is also interesting. In the Institute of Plant Physiology of the CPR Academy of Sciences, it was established that nonfruit-bearing pods of rape are connected with insufficient feeding after flowering, low temperatures and virus diseases. It appeared that some rational feeding leading to a redistribution between the individual organs and a change in the rate of movement of assimilates eliminates, or, in any case, decreases, the number of nonfruit-bearing pods of rape. Obviously, the use of physiologically active substances also shows a positive effect here.

Investigations on growth substances, including gibberellin, are being developed more and more widely in many institutes and higher educational institutions in the CPR: in the Institute of Plant Physiology of the Academy of Sciences (Professor Lo Shih-wie) at the Departments of Plant Physiology and Laboratories of Plant Physiology of Peking (Professor Tank P'ieh-sun and scientific colleague, Shao Li-mei), Teintsin (Professor Ts'ui Ch'eng), and Shanghai (lecturer Shie Ying-lu) Universities, in the Pedagogical Institute, in the Institute of Agronomy and Selection of the Academy of Agricultural Sciences (Peking), in the Biological-Soil Institute of the Northwest Branch of the CPR Academy of Sciences (director Professor Yü Hung-ts'ing) and others. At the same time much attention is being given to the discovery of new growth substances with a high physiological activity.

Of no less interest are the projected works on radiobiology in the Institute of Plant Physiology of the CPR Academy of Science (Professor Lo Tsung-lo), and the application of radioactive substances with the goal of bettering the field characteristics of plants for the creation of highly productive crops. Two questions should be resolved here: 1) the physiological nature of plants under the effect of the action of radioactive rays, and 2) the physiological nature of varied response of plants to radioactive rays: a) the morphological and anatomical changes, b) the histochemical changes of the protoplasm, and c) the biochemical changes.

The study of heredity, plant selection and microorganisms under the effect of radioactive rays is also being carried out in Shanghai University (Department of Genetics).

Biophysical investigations have been organized (Peking University, Shanghai University, and others) with the goal being the application of the achievements of physics in the practice of agriculture and in the scientific explanation of the experiment of the progressive farmers.

Microbiological investigations have received broad development at the Institute of Plant Physiology of the CPR Academy of Sciences (Professor Shen Shang-chung), at Shanghai University, at the Shanghai Pedagogical Institute, and at others. These works lead to the close cooperation and contact with factories and medical institutes. They have developed in different directions: 1) the use of antibiotics, 2) the extraction of vegetable oil from rice straw as a result of fermentation, 3) the obtaining of acetone and butyl alcohol on fermentation, and 4) the effect of radioactive rays on microorganisms. In many of the higher educational institutions, which have departments of microbiology, and also in the scientific-research institutes (Soils in Nanking, Institute of Agronomy of the Academy of Agricultural Science) which have small factories for the production of bacterial fertilizers which are fairly widely used in agriculture..

Very valuable investigations have been carried out by the Soil Institute of the CPR Academy of Sciences in Nanking (Professor Li and others) on the study of the change of soil fertility in connection with deep plowing, layered application of organic, mineral and microfertilizers, and also bacterial fertilizers, and with the use of irrigation and other agricultural measures. At the same time the Institute is developing the practical questions of a rational system of plant feeding and other means of increasing the yield of agricultural crops. The Institute is also carrying on investigations on the study of soil resources, deciding a number of important theoretical questions (the genesis of soils) and developing new methods of use for isotopes under laboratory conditions.

The broad and all encompassing investigations of the Institute of Agronomy and Selection of the Academy of Agricultural Sciences (Peking) merit great attention and a high rating, both on the scientific generalization of the "big leap" in agriculture and in the treatment of the theoretical bases of increasing the productivity of plants. Along with this, the Institute has organized a beautiful display of its most important work. The broad investigations of the Botanical Institute of the CPR Academy of Sciences in Kwangchou (director, Professor Ch'ieng Hua-yün) are interesting, in particular, those on the physiology of tropical and subtropical crops (Professor To Chiung-yen), and the special work on the culture of tea plants in the Tea Institute of the Academy of Agricultural Sciences in Hichou.

Finally, in all of the botanical institutes, botanical gardens, and at the corresponding departments of the higher educational institutions, investigations are organized to discover useful wild plants (fiber, medicinal, rubber, technical, and others) for use in agriculture and industry.

Of course, only a small part of the many-sided work of the Chinese biologists is given in this article. However, even from this short paper, one can bet an idea of the dimensions and significance of biological investigations carried out in the CPR in 1958, the year of the big leap in agriculture.

4. What Is Useful in the Experiment of the Chinese Progressive Farmers and Scientists That Can Be Used for Agriculture in Our Country and for Improving the Scientific Work of Soviet Scientists and Biologists?

It seems that first of all it would be feasible to organize experiments on the study, in our conditions, of the effectiveness of the combination of deep plowing and the layered application of large doses of organic fertilizers and nutrients. Very likely these measures would lead to a sharp increase in the productivity of the most valuable crops. It is necessary here to pay particular attention to the study of the physiological role of large doses of organic fertilizers.

No less valuable would be the study of the role and importance of deep plowing (35-40 cm) for rice. The root system for rice, as specialists know, is usually in the very uppermost layer of the soil. Deep plowing, undoubtedly would lead to the start of deeper penetration for the roots and the thickening of the roots so that they, in turn, strengthen the plant itself.

An experiment on the irrigation of rice would be very valuable. In 1958 in the CPR, a new method of irrigating rice without a permanent deep layer of water and with a time of drying during flower formation was used. The use of this method of irrigation made it possible to decrease significantly the irrigation norm of water, protect the rice from breakage and obtain high yields of grain.

Also, it is necessary to consider a positive experiment on the use of herbicides for the battle with weeds on rice areas, especially with such harmful weeds as rice bunting.

Usually it is very difficult with rice to obtain an even, strong, and consistent stand of sprouts. The main reason for this is the variability in the quality of the seeds, which are unequally ripened. Here it is also very

important to establish experiments on the use of physiologically active substances. With their help, apparently one can approach a more equal degree of ripeness and obtain full value rice seeds and, therefore, even sprouts. Obviously by this same means one can get equal and more rapid ripeness of legumes and improve the quality of rape seeds and many other crops.

Great achievements were made in the CPR in the battle with breakage of grain and other crops in conditions of very high yields.

As a result, a new system of agricultural methods using physiologically active substances and the introduction into agricultural practice of highly productive nonbreaking varieties has been realized. It is also necessary to consider this experiment for which it is necessary to organize a complex study of the questions of plant breakage.

The problem of "photosynthesis and yields", successfully developed recently in the CPR as a result of the "big leap" in agriculture is of great theoretical and practical significance. In this connection, it would be very expedient to establish special experiments for the complex study of this problem with an advanced system of agricultural methods with the goal of the establishment of the actuality of a significantly higher coefficient of use of the sun's energy by the plants in each group.

Finally, it would be very useful and valuable for us to disseminate the scientific-organization experiment of the Chinese scientists on the generalization of the "big leap" in agriculture. At the call of the Communist Party of China, literally all scientific-research institutes, institutions, and higher educational institutions of the CPR, as we have already stated, included themselves in this work. It worked on a wide front as an enormous collective, using complex methods of investigation. Only by such means was it possible to resolve so rapidly and accurately the important tasks of agriculture.

In 1959 our Chinese friends continue to work with great enthusiasm to fulfill the impressive tasks set up by the Communist Party and the Government of the CPR. We sincerely hope that they crown this year with a new and still "bigger leap" in agriculture and in science and in building socialism.

CHRONICLE

RESOLUTION OF THE CONFERENCE ON THE PHYSIOLOGY OF PLANT HARDINESS, ADOPTED MARCH 7, 1959

One of the most important tasks set forth by the XXI Congress of the Communist Party of the USSR (CPSU) is the increase, in every way, of the yields of agricultural plants with the aim of satisfying the growing needs of the population for food products and of industry for raw materials.

The successful fulfillment of this large and important task of the economy depends, to a significant degree, on the development of effective means of fighting death, injury and decrease in yields of agricultural plants as a result of frost, drought and saliferous soils, because an unfavorable effect from at least one of these factors exists in practically all regions of the USSR.

In connection with this, there are also important and responsible tasks facing the phytophysiologists on further broadening and deepening investigations on the hardiness of plants with the goal of establishing the scientific bases of a number of measures providing for the adaptation and sufficiently high productivity of plants in unfavorable external conditions.

One must welcome the initiative of the Institute of Plant Physiology of the USSR Academy of Sciences, the M. V. Lomonosov Biological Soils Faculty of Moscow State University and the Scientific-Technical Soviet of the USSR Ministry of Higher Education on the call for the Conference on the Physiology of Plant Hardiness. The representatives of 95 institutions with reports, and 33 institutions without reports took part in the work of the Conference, which was held in Moscow from March 3 to 7, 1959. It was explained that questions of plant hardiness would be developed in all of the republics of the Soviet Union. The institutes of the USSR Academy of Sciences and their branches (83 reports), agricultural institutions (63 reports) and the VUZ's (46 reports) are working on this problem. Representatives of the peoples' democracies took part in the work of the Conference. In all, there were 198 reports, including 98 on winter resistance of plants, 65 on drought resistance and 35 on salt resistance.

The Conference aided in the mutual acquaintance of physiologists of the Soviet Union and the friendly democratic republics with the status of scientific investigations, and aided the collective development of the prospects for future scientific work in the area of plant hardiness.

The conference noted with satisfaction that because of the creation of an artificial climate in the K. A. Timiryazev Institute of Plant Physiology of the USSR Academy of Sciences, the possibility for the development of investigations on the physiology of plant hardiness was broadened. Effectively using these possibilities, the K. A. Timiryazev Institute of Plant Physiology has already obtained important results having significant importance for further development of the theory of plant hardiness. The Conference also noted the successes in plant hardiness in a number of scientific institutions of the Ukraine Academy of Agricultural Sciences, in the Academy of Sciences in Latvia, Estonia, Uzbekistan, Turkmenia, Kazakhstan, Kirgizia, Armenian Soviet Republic, in the Bashkir, Karelian and Moldavian branches of the USSR Academy of Sciences, in the V. L. Komarov Botanical Institute of the USSR Academy of Sciences, in the main Botanical Garden of the USSR Academy of Sciences, in VIR, VNIIChISK, VIUAA, Moscow State University, Kiev State University, in the Timiryazev Agricultural Academy and in other higher educational institutions.

I. Winter Resistance of Plants

The Conference noted that a great deal of work in the Soviet Union is being carried out on the problem of the physiology of winter resistance of plants. By theoretical investigations on winter resistance of plants in the laboratory of the K. A. Timiryazev Institute of Plant Physiology, the prospect of work on increasing the

frost resistance of plants was shown. The physiological principals, on the basis of which sharp increases in the frost resistance of plants (several of which were not killed even at—195°) have been attained already under laboratory conditions, were shown. A further task is to find a means of increasing the winter resistance of agricultural crops in the field and in gardens. For this, in addition to deeper theoretical work, the physiologists must force the development of a system of practical measures.

A great deal of work has been done by physiologists on explaining physiological and biochemical changes (in the carbohydrate composition, the intensity of respiration, the activity of enzymes, the condition of colloids, etc.) that take place in plants during the cold period of the year. On this basis, methods of biological control of the condition of winter-resistant plants and of the degree of their injury are being developed. In order to establish the reasons for winter kill of plants, physiologists are giving a great deal of attention to the study of the effect of external factors: the temperature, light and water regime and also the different nutrition of plants. It has already been explained that the plants frost resistance depends on the age, development, intensity of the growth processes and the shift into the quiescent condition.

On the basis of the study of the overwintering of winter crops in the various regions of the Soviet Union, means of increasing the frost resistance of plants both by technical-agriculture methods and by means of selection have been developed.

Many physiologists worked on the problem of increasing resistance to cold of southern plants, both in connection with the advancement in the sowing of corn and other thermophilic crops in the colder regions and with the necessity of planting them earlier to increase the yields. Besides the theoretical investigations, the development of practical means of strengthening the resistance of plantings to chilling must also be noted. Those which are considered practical among them are preplanting temperature hardening of the seeds, preplanting treatment of the planted material with salt solutions, including microelements, and also the use of fungicides for overcoming the pathogens of the cold soil and the selection of more resistant forms.

The broad development of investigations on the winter resistance of woody plants (fruit trees, grape, and subtropical crops) in the Soviet Union has been started, in connection with the death of fruit plantings during the cold period of the year. A great deal of attention has been turned to an explanation of the effect of the conditions of the preceding summer on resistance during the cold period of the year. Besides this, physiological and biochemical changes connected with the favorable overwintering of plantings have been established and practical recommendations have been developed on increasing winter resistance.

The Conference, noting the results already achieved, thinks that the experiments carried out still do not completely answer the needs of agronomy and that the results obtained are not always being put into practice widely and rapidly.

The Conference recommends that attention be paid to further investigation on winter resistance of plants, with the development of the following problems.

1) The reasons for winter kill of seedlings and plantings in the different regions of Soviet Union. It is necessary to study in more detail the effect of frost kill, frost heave, winter drying and to find means to reduce them.

2) The effect of external factors and technical agriculture methods on the winter resistance of different crops. In connection with strengthening the chemization of agronomy, it is necessary to use fully fertilizers and microelements in order to attain favorable overwintering of plants.

3) To study the frost resistance of an assortment of overwintering perennial grass and fruit and subtropical crops with the goal of determining their more correct range. A means must be found of accelerating the discovery of highly frost-resistant forms.

4) To strengthen the development of a theory of frost resistance of plants by means of the study of the effect of hardening and the physiological features of the frost-resistant forms. When studying the period of quiescence, attention must be turned to the development of methods of simultaneously introducing plants into the quiescent condition and lengthening its duration. New means must be found of increasing the winter resistance of plants by means of the action of chemical and physical factors, in particular, by means of preplanting treatment of seeds and planted material. The development of methods must be strengthened in the battle with spring and autumn frosts.

5) To strengthen the investigation of winter grain crops in the direction of:

a) the study of the features of the formation of frost resistance and its dynamics during the cold period of the year for winter wheat and rye in connection with their staged development, age condition of the plants, stages of organogenesis, conditions of cultivating and different agricultural methods;

b) the development of new methods of diagnostics of frost resistance for primary selection material with the goal of discovering highly winter-resistant varieties of winter crops. The development of methods of biological control of the condition and degree of damage of winter plants.

6) To broaden investigations on winter resistance of perennial grasses and to explain its dependence on the staged development, the light regime, the continuance of the process of hardening, the age of the plants, the number of mowings, and the environmental conditions. Methods must be developed of increasing the winter resistance of perennial grasses with the help of the creation of the appropriate agricultural conditions for their cultivation with the application of various macro-and microfertilizers.

7) To turn attention in investigations on the winter resistance of plants to the study of the annual rhythm of metabolism, growth, and development of trees and bushes of different ages with the calculation of their relationship to the features of the climate of the separate zones of the country; to develop methods of increasing frost resistance and its early diagnosis for fruit berry and ornamental crops.

8) To strengthen the development of the physiological foundations of agricultural techniques resulting in an increase in the winter resistance of grape plantings (questions of plant nutrition, water regime, formation, shipping, etc.). The possibility must be established of extending the area of the unsheltered culture of grape. Methods must be developed of diagnostics of frost resistance of varieties of grape in the early stages of the selection process and means of chemical treatment of plants with a goal of protecting grape bushes from spring frosts. Studies on the physiological effect of the wildings on the winter resistance of grape must be made.

9) To expand work on the winter resistance of subtropical and southern crops, paying particular attention here to a critical synthesis of accumulated data and the development of an ordinary theory of winter resistance of southern crops with regard to their adaptive evolution. Work must be strengthened on the amelioration of climate for easing the conditions of overwintering of subtropical crops with the use of new achievements in energetics and natural heat resources, particularly thermal water, recently uncovered in the zone of the humid subtropics of western Georgia.

To accelerate the development of a method of protecting perennial southern crops from frost and early frost by means of spraying the plants with solutions developed by the Scientific-Research Institute of Viticulture, Wine Making and Fruit Industry of MSKh, Armenian SSR.

10) To force the study of the theoretical bases of cold resistance of thermophilic crops (corn, cotton, vegetables) and the development of effective means of preservation of seedlings through periods of chilling, particularly in the first periods of growth of the plants (hardening by cold and changing temperatures, the use of microelements, the treatment of seeds and root zone layers of the soil with fungicides, rationalization of agricultural techniques). Attention must be turned to the development of methods of early diagnosis of the degree of cold resistance of plants by indicators of the physiological and biochemical condition of the plants.

II. Drought Resistance of Plants

The Conference noted that broad investigations have been carried out on the physiology of the drought resistance of plants in the Soviet Union which have accumulated a great deal of experimental material and have made a number of valuable generalizations. The reason for injury to plants in conditions of drought, and also the means of adapting them to soil and atmospheric dryness, have been explained. The leading colloid chemical changes and physiological processes which lie at the base of the resistance of cells to dehydration and overheating have been established. As a result, practical methods have been presented for the management of plants so as to increase their drought resistance and productivity. Methods of diagnosing drought resistance of cultured plants have been developed.

However, in light of the history-making resolution of the XXI Congress of the CPSU on the future improvement of yields, it is necessary to strengthen the theoretical investigations, which can serve as a basis for the development of a number of new methods of diagnosis and increase drought resistance with broad practical application.

The Conference considers that:

1) When the theoretical problems are resolved, the main efforts must be directed at the study of the protective-adaptive reactions of plants as an entire, developed system. Further and deeper study of the characteristics and changes under the effect of drought in the physiology of metabolism and the colloid-chemical characteristics of the protoplasm is necessary. One of the most important tasks is the study of the interaction between the colloid-chemical characteristics and the biochemical processes of drought-resistant plants. A significant place in this work must also be taken by the study of the connection of the physiological processes with the anatomical morphological features of the plants. These investigations must be carried out on a new level with an explanation not only of the microscopic, but also of the submicroscopic, structure of the cell.

2) Of particular importance are the questions of the interrelation of the processes of photosynthesis, growth and development which determine the yield and its quality in conditions of drought. Further study is necessary on the characteristics of the protoplasm, water regime, and metabolism for the basic group of agricultural plants and also for fruit crops under various types of drought.

It is necessary to explain: a) the effect of microelements on the drought and heat resistance of the most important agricultural plants in relation to their stages and phases of development; b) the movement of plastic substances in the plants as a single whole in conditions of various types of drought; c) the characteristics of metabolism in the root system with its absorbing and secretory functions under conditions of soil and atmospheric drought.

The Conference notes the necessity of further development of methods in the study of the forms of water in plants, because this important question has not received complete development at the present time.

3) One of the important practical aims is the study of drought resistance, that is, the ability to carry over through dehydration and overheating of a number of varieties of cultured plants. The task is complicated by the fact that it must be resolved in a different manner for the different regions and for different types of drought.

4) It is necessary to pay attention to the further development of the theory of heat resistance of plants for generalizing the methods in the battle with the different heat injuries and deviations from the norm (wilting, degeneration of tubers, anomalies of the growth of affected plants, etc.).

The effect of an increase in temperature with the simultaneous complex action of other factors of the environment (dehydration, anaerobiosis, different lighting, soil salt, infection with parasites and diseases) must be studied.

5) It is necessary to turn attention to the further development of methods of diagnostics of the resistance to overheating and dehydration of cultured plants at the various stages of ontogeny.

6) The study of the physiology of preplanting hardening of plants and the effect of other methods of increasing drought resistance (the use of microelements, sequence fertilizers, etc.) and their effective interaction with each other must be strengthened.

7) It is necessary to study the subsequent effect of drought, dehydration and overheating on the following generations, keeping in mind the colloid-chemical characteristics, physiological and biochemical processes, certain forming and hereditary characteristics of high drought resistance, and also on the physiological and colloid-chemical features of the original forms of the hybrids showing high productivity and drought resistance.

8) It is necessary to study the internal interrelationships of crop plants in sowing in the different stages of their individual development under different types of drought, and also the resistance to drought of natural phytogroups and to develop methods to increase productivity.

9) Based on a line of technical agriculture methods, the increase in the concentration of water in the soil must be considered. The plant physiologist must help technical agriculture to appraise which type of soil treatment and fertilizing of sowings and plantings is best for drought resistance and productivity of plants.

10) In connection with selection work, plant physiology must develop the premises for directing selection toward creation of highly productive and at the same time drought-resistant varieties. When this question is resolved, preplanting hardening to drought must be tested as a method of growing plants necessarily bringing about the selection and crossing of plants resistant to drought, against a background of moderate soil and

atmospheric drought. The combination of high productivity and significant drought resistance is possible because the changes of plants on hardening move toward a strengthening of metabolism and growth processes, which also makes it possible to hope to obtain constant drought-resistant varieties of high productivity.

11) Using the necessary assortment of methods of investigation for the study of drought resistance, plant physiologists must develop new, original and simple methods for the field diagnosis of drought resistance in plants.

It is necessary to specify and unify the existing methods of the study of the water regime and the resistance of plants to various types of drought, and also to develop new methods of investigation (in particular, of study of the root system of plants, including the activity of the root hairs, and also the measurement of the rate of water flow in the plant, etc.).

III. Salt Resistance of Plants

The Conference considers that the battle with salinity of soils, which does great damage to agriculture by decreasing the yields of crop plants and lowering their quality, must be accomplished both by means of amelioration and by means of increasing the salt resistance of plants.

In the last decade, the anatomical-morphological and physiological changes of plants have been relatively well studied from the point of view of adapting them in the process of ontogeny to the conditions of saliferous soils. With this, several of the characteristics of salt resistance in plants were established. This served as a basis for the development of a number of methods of increasing the salt resistance of plants. Along with this, it was explained that this characteristic, acquired during ontogeny, is fixed in the descendents, which makes it possible to develop the method of perennial cultivation of plants in salty conditions with the goal of increasing their salt resistance and productivity.

In recent years investigations were started in the area of the study of the mechanism of the absorption of salts and the transfer of ions, and also the mechanism of the toxic action of salts on the plant organism and its answering reaction to the action of the salt, with the calculation of the type of salinity of the soil.

While acknowledging certain achievements in the development of the problem of the salt resistance of plants, still it must be thought that the theoretical investigations in this direction are insufficiently broad and do not fully correspond to the level of modern plant physiology. Up to the present time, the theoretical generalization on the physiology of salt resistance of plants, uniting the unconnected ideas on the reactions of various biological groups of plants to various conditions of salty soil, has been inadequately developed.

It must also be considered that the results of the theoretical investigations up to the present time have not been used to any significant degree in the practice of agriculture, as a result of which the recommended methods of increasing the salt resistance of plants and measures increasing the yields on saliferous soils are not going out beyond production testing.

Among the important tasks for future investigations, the following must be considered:

1) The study of the nature of salt resistance of plants and the mechanism of the toxic action of salts on the plant cell. The change of the physical-chemical and visible characteristics of the cell and the cell organs as a result of the action of the salt. There is significant interest in the study of the successive conversion of substances with the aim of establishing and explaining the intermediate products of metabolism which have a toxic effect on the plant organism.

2) Considering that at the present time we still do not have sufficient information on the mechanism of the action of salts on the physiological processes of plants, particularly on photosynthesis and respiration (on which the intensity and relations of the plants depend), it is necessary to study the effect of salts and ions both on the separate intermediate reactions from which photosynthesis and respiration are put together, and also on enzymes taking part in these processes.

3) It is necessary to carry out investigations directed toward explaining specifically the metabolism of plants on various types of salty soil. For this, a study of the proteins, which enter into an interaction with the salts, is very important.

4) To broaden investigations on the water exchange and water regime of plants, to give a basis for national irrigation of agricultural crops under salty conditions.

5) To carry out further investigation on the assimilation of individual salt elements (N, P, K and others) into the plants in relationship to the type and degree of saltiness of the soil based on the effect of saltiness on the involvement of the given elements in metabolism.

6) Very important is the study of the physiology of the individual organs (root, stem, leaf) in conditions of saltiness with the aim of expalining their role in the resistance of the plant as a whole.

7) To broaden investigations on the physiology of salt resistance of cultured plants both in ontogeny and in a number of generations.

8) It is necessary to continue the development of methods of diagnostics of salt resistance applicable to the individual crops and to various types of salinity.

9) The increase in the productivity of crop plants grown on saliferous soils can be successfully attained only with the correct and, moreover, simultaneous, combination of measures of amelioration of the saltiness of soils with the selection and development of salt-resistant varieties of plants. After this, it is necessary in the future to continue investigations in the area of salt resistance of individual crops and in the area of an explanation of their variety features in the application to certain types of salinity of soils. In resolving these tasks, selection will have a big role in salt resistance.

10) In investigations of the salt resistance of plants, it is necessary to consider not only the degree of mineralization of the ground water, but also the presence of organic and mineral substances formed as a result of the activity of microorganisms.

11) The Conference considers that the basic measure in the utilization of medium and strongly saliferous soils is their amelioration. Together with this, it is also necessary to use, widely, biological methods of increasing the agronomical salt resistance of plants on saliferous soils. For this, the following can be recommended:

a) To investigate widely on the experimental stations located on saliferous soils the methods of preplanting treatment of seeds with salt solutions.

b) To test on these same experimental stations the effectiveness of the action of such microelements as zinc, boron, copper, and also salts of iron, on the cultivation of agricultural crops on soils of weak and medium salinity. The methods of using the microelements could be: preplanting soaking of the seeds, foliar applications, application of the microelements to the soil.

c) To ask the Turkmensk Academy of Sciences to establish on takyrs, subject to appropriation in connection with the construction of the Kara-Kums canal, production experiments on the use of vitamins for increasing cotton yields.

d) In view of the fact that the ability of plants to overcome unfavorable conditions of salinity depends to a significant degree on their physiological condition, on the conditions of fertility of the soil and on the level of agricultural techniques, the study of a system of crop rotation for saliferous soils, the spacing and distribution of fertilizers in the given crop rotations, and also the application of rational methods of irrigation are considered the most important tasks of the scientific research work in this direction.

e) It is necessary to broaden investigations on the questions of the selection of crops resistant to the alkalinity remaining after amelioration.

f) It is expedient because of the differences of soils and regions of the USSR to establish differential scales of permissible toxic concentrations of salts in the soil for agricultural plants and also to develop a classification of soil salinity by degree of salinity in relation to the type of salinity.

IV. Organization Measures

1) For the more successful development of the problem of increasing the resistance of plants to unfavorable external conditions, it is necessary to raise the level of theoretical investigations. For this, the new achievements in the neighboring sciences must be used: in biochemistry, biophysics, agrochemistry, organic chemistry, physical chemistry, physics, etc.

The equipping of physiological laboratories with the necessary equipment can be of great help in the matter of improving the work. To ask the Presidium of the USSR Academy of Sciences to appeal to the Gosplan of the USSR with a petition on the organization in the Soviet Union of refrigeration units according to the technical assignment of the K. A. Timiryazev Institute of Plant Physiology. To ask, also, the Leningrad Sovnarkhoz to begin an issue of semiconducting microrefrigerators with the corresponding electrical and measuring apparatus at the Leningrad experimental factory and OKTB semiconducting and ultrasonic instruments.

3) To recommend the wider use of modern methods of investigation (climatization, radioactive and heavy isotopes, chromotography, optical methods, etc.). To ask the editors of the journal "Fiziologiya rastenii" to expand information on new instruments and on methods of investigation.

4) Because the problem of resistance of plants can be successfully solved by the cooperative strengthening of the workers of a number of related disciplines, to recommend investigations together with technical agriculture workers, selection workers geneticists, soil scientists and other specialists. Because the selection of resistant forms and the development of technical agriculture methods must be carried out in relation to concrete features of the regions, to ask the USSR Ministry of Agriculture and VASKhNIL to organize laboratories of plant physiology and biochemistry in branch institutes and on selection experiment stations.

5) To turn the attention of all scientific institutions and workers on plant hardiness to the necessity of rapid and broad application of the results of scientific investigations in agricultural production by means of taking part in the development of corresponding measures. It is necessary to generalize the experiments of the forerunners in agriculture that have already been achieved. To carry out production tests of the methods named for application: preplanting hardening of seeds to drought and to salinity, hardening to cold of seeds of thermophilic crops by use of cold and shifting temperatures and the treatment of their seeds and soils with fungicides, increasing resistance by means of the application of microelements, etc.

To recommend the publication of a number of scientific-popular brochures on the methods of increasing the resistance of plants.

6) To accomplish the coordination of work on the resistance of plants and the unification of methods of investigation at periodically occurring All-Union conferences. It would be expedient to have the next Conference on the Physiology of Plant Hardiness in 1964. Prior to this, in order to coordinate experiments, to meet at lower level conferences which would convene in various places in the Soviet Union.

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci.-Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiya	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiya	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Émbriol.	Arkhiv Anatomii Gistologii i Émbriologii	Archives of Antomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiya	Biokhimiya	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiya Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Byull. Éksptl. Biol. i Med.	Byulleten Éksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Byull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Byulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Éksptl. Khirurg.	Éksperimentalnaya Khirurgiya	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiya i Toksikologiya	Pharmacology and Toxicology
Farmatsiya	Farmatsiya	Pharmacy
Fiziol. Rastenii	Fiziologiya Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenov Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariya	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiya Tikhookeanskogo N. I. Instituta Rybnogo Khozyaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiya	Khirurgiya	Surgery
Klin. Med.	Klinicheskaya Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaya Parazitologiya i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaya Radiologiya	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiya	Mikrobiologiya	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhyat. i Psikhogig.	Nevropatologiya, Psikhyatriya i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediya, Travmatologiya i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriya	Pediatriya	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Éndokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniya Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaya Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journal
Stomatologiya	Stomatologiya	Stomatology

*BRITISH-AMERICAN TRANSLITERATION SYSTEM.

(continued)

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Abbreviation	Journal	Translation
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriya	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiya	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiya	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiya, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoyuz. Gidrobiol. Obshchestva	Trudy Vsesoyuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoyuz. Inst. Eksptl. Med.	Trudy Vsesoyuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiya	Urologiya	Urology
Uspekhi Biokhimiya	Uspekhi Biokhimiya	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiya	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriya Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universitera, Seriya Biologii i Pochvovedeniya	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiya	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariya	Veterinariya	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniya	Voprosy Pitaniya	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaya Laboratoriya	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiya	Journal of General Biology
Zhur. Vysshei Nerv. Deyatel.	Zhurnal Vysshei Nervnoi Deyatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

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